A STUDY TO CORRELATE SERUM MAGNESIUM, SERUM CALCIUM, HbA1c AND NERVE CONDUCTION PARAMETERS IN TYPE 2 DIABETES MELLITUS

Dissertation submitted to

THE TAMILNADU Dr. M.G.R. MEDICAL UNIVERSITY
CHENNAI – 600 032

In partial fulfillment of the requirement for the degree of
Doctor of Medicine in Physiology (Branch V)

M.D. (PHYSIOLOGY)
APRIL – 2017

DEPARTMENT OF PHYSIOLOGY
TIRUNELVELI MEDICAL COLLEGE
TIRUNELVELI – 627 011.

CERTIFICATE

This is to certify that the dissertation entitled, **"A STUDY TO CORRELATE SERUM MAGNESIUM, SERUM CALCIUM, HbA1c AND NERVE CONDUCTION PARAMETERS IN TYPE 2 DIABETES MELLITUS"** done by Dr. I.J.V. PRADEEP VAIZ, T, post graduate in PHYSIOLOGY (2014-2017), is a bonafide research work carried out under our direct supervision and guidance and is submitted to The Tamilnadu Dr. M.G.R. Medical University, Chennai, for M.D. Degree Examination in Physiology (Branch V), to be held in April 2017.

Dr. Ratna Manjushree Jayaraman M.D., DCH.,
Associate Professor and Head,
Department of Physiology,
Tirunelveli medical college,
Tirunelveli – 11.

Dr. K. Sithy Athiya Munavarah M.D.,
Dean,
Tirunelveli medical college,
Tirunelveli - 11.

ENDORSEMENT BY THE GUIDE

This is to certify that the dissertation entitled, **"A STUDY TO CORRELATE SERUM MAGNESIUM, SERUM CALCIUM, HbA1c AND NERVE CONDUCTION PARAMETERS IN TYPE 2 DIABETES MELLITUS''** is a bonafide research work carried out by Dr. I.J.V. PRADEEP VAIZ, T, in the Department of Physiology, Tirunelveli Medical College Hospital, Tirunelveli – 11 under my direct guidance and supervision in partial fulfillment of the requirement for the award of the degree of MD in PHYSIOLOGY (Branch – V) in April 2017.

GUIDE:

Dr. B. SUJATHA M.D., D.A.
Associate Professor
Department of Physiology
Tirunelveli Medical College
Tirunelveli – 11.

DECLARATION

I solemnly declare that the dissertation entitled **"A STUDY TO CORRELATE SERUM MAGNESIUM, SERUM CALCIUM, HbA1c AND NERVE CONDUCTION PARAMETERS IN TYPE 2 DIABETES MELLITUS''** is done by me at Tirunelveli Medical College Hospital, Tirunelveli.

The dissertation is submitted to The Tamilnadu Dr. M.G.R. Medical University towards the partial fulfillment of the requirement for the award of M.D. Degree (Branch V) in Physiology.

Place : Tirunelveli

Date :

Dr. I.J.V. PRADEEP VAIZ, T

Postgraduate Student

M.D. (Physiology)

Department of Physiology

Tirunelveli Medical College

Tirunelveli.

TIRUNELVELI MEDICAL COLLEGE

INSTITUTIONAL RESEARCH ETHICS COMMITTEE
TIRUNELVELI, STATE OF TAMILNADU, SOUTH INDIA PIN 627011
91-462-2572733-EXT; 91-462-2572944; 91-462-2579785; 91-462-2572611-16
online@tvmc.ac.in, tirec@tvmc.ac.in; www.tvmc.ac.in

CERTIFICATE OF REGISTRATION & APPROVAL OF THE TIREC

REF NO:686/PHYSIO/2015

PROTOCOL TITLE: STUDY TO CORRELATE SERUM MAGNESIUM AND CALCIUM LEVELS, HbAIC AND NERVE CONDUCTION STUDIES IN NEWLY DIAGNOSED TYPE II DIABETES MELLITUS.

PRINCIPAL INVESTIGATOR: Dr. I.J.V. PRADEEP VAIZ, MBBS.,

DESIGNATION OF PRINCIPAL INVESTIGATOR: POST GRADUATE IN PHYSIOLOGY
DEPARTMENT & INSTITUTION: TIRUNELVELI MEDICAL COLLEGE, TIRUNELVELI

Dear , Dr. I.J.V Pradeep Vaiz, MBBS., The Tirunelveli Medical College Institutional Ethics Committee (TIREC) reviewed and discussed your application during the IEC meeting held on 10.06.2015.

THE FOLLOWING DOCUMENTS WERE REVIEWED AND APPROVED

1. TIREC Application Form ☐
2. Study Protocol ☐
3. Department Research Committee Approval ☐
4. Patient Information Document and Consent Form in English and Vernacular Language ☐
5. Investigator's Brochure ☐
6. Proposed Methods for Patient Accrual Proposed ☐
7. Curriculum Vitae of the Principal Investigator ☐
8. Insurance /Compensation Policy ☐
9. Investigator's Agreement with Sponsor ☐
10. Investigator's Undertaking ☐
11. DCGI/DGFT approval ☐
12. Clinical Trial Agreement (CTA) ☐
13. Memorandum of Understanding (MOU)/Material Transfer Agreement (MTA) ☐
14. Clinical Trials Registry-India (CTRI) Registration ☐

THE PROTOCOL IS APPROVED IN ITS PRESENTED FORM ON THE FOLLOWING CONDITIONS

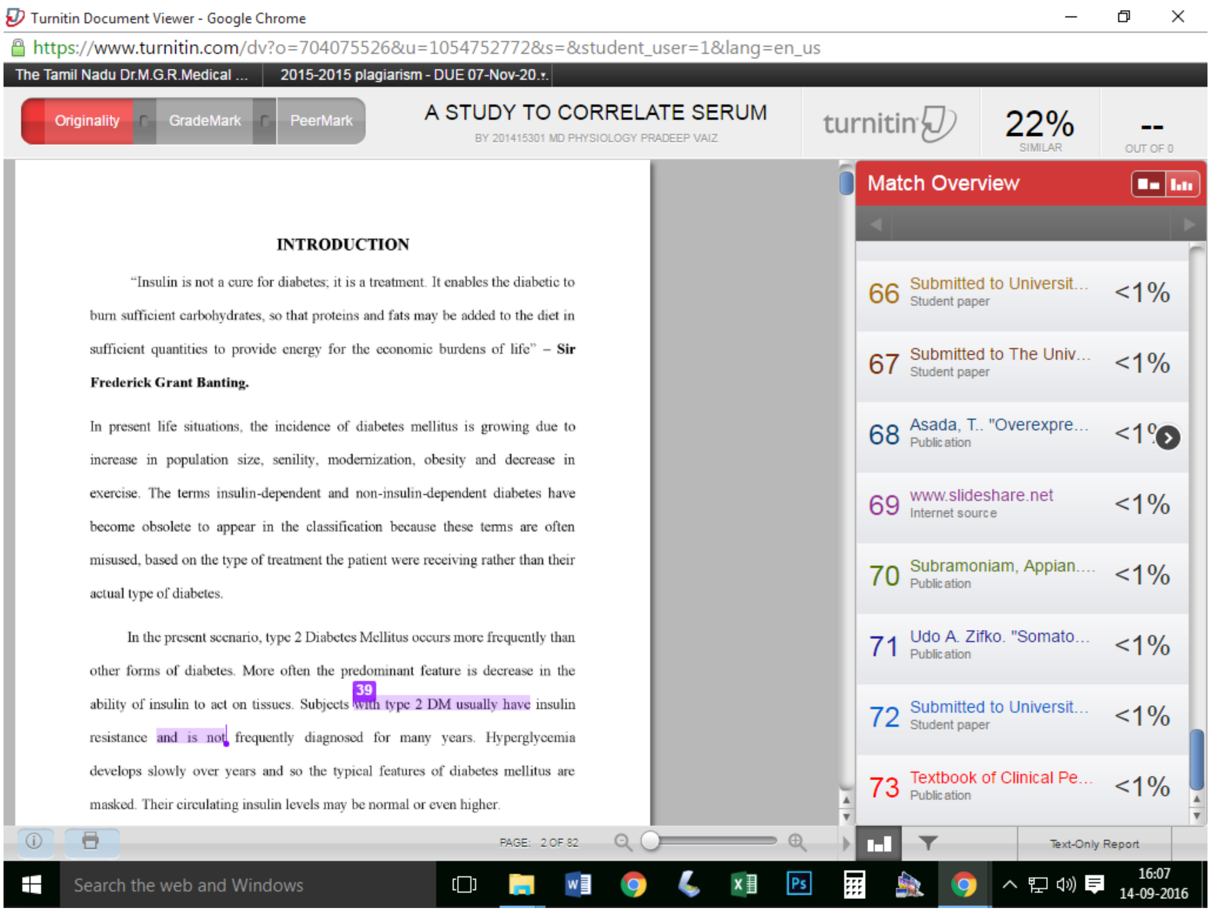
1. The approval is valid for a period of 2 year/s or duration of project whichever is later
2. The date of commencement of study should be informed
3. A written request should be submitted 3weeks before for renewal / extension of the validity
4. An annual status report should be submitted.
5. The TIREC will monitor the study
6. At the time of PI's retirement/leaving the institute, the study responsibility should be transferred to a person cleared by HOD
7. The PI should report to TIREC within 7 days of the occurrence of the SAE. If the SAE is Death, the Bioethics Cell should receive the SAE reporting form within 24 hours of the occurrence.
8. In the events of any protocol amendments, TIREC must be informed and the amendments should be highlighted in clear terms as follows:
   a. The exact alteration/amendment should be specified and indicated where the amendment occurred in the original project. (Page no. Clause no. etc.)
   b. The PI must comment how proposed amendment will affect the ongoing trial. Alteration in the budgetary status, staff requirement should be clearly indicated and the revised budget form should be submitted.
   c. If the amendments require a change in the consent form, the copy of revised Consent Form should be submitted to Ethics Committee for approval. If the amendment demands a re-look at the toxicity or side effects to patients, the same should be documented.
   d. If there are any amendments in the trial design, these must be incorporated in the protocol, and other study documents. These revised documents should be submitted for approval of the IEC, only then can they be implemented.
   e. Approval for amendment changes must be obtained prior to implementation of changes.
   f. The amendment is unlikely to be approved by the IEC unless all the above information is provided.
   g. Any deviation/violation/waiver in the protocol must be informed.

STANDS APPROVED UNDER SEAL

Dr.K.Shantaraman MD
Registrar, TIREC
Tirunelveli Medical College, Tirunelveli – 627011
State of Tamilnadu, South India

TIRUNELVELI MEDICAL COLLEGE
TAMILNADU INDIA
TIRUNELVELI

Dr.V.Ramasubramanian MD DM
Member Secretary, TIREC
Tirunelveli Medical College, Tirunelveli – 627011
State of Tamilnadu, South India


Turnitin Document Viewer - Google Chrome

https://www.turnitin.com/dv?o=704075526&u=1054752772&s=&student_user=1&lang=en_us

The Tamil Nadu Dr.M.G.R.Medical ... | 2015-2015 plagiarism - DUE 07-Nov-20...

Originality | GradeMark | PeerMark

A STUDY TO CORRELATE SERUM

BY 201415301 MD PHYSIOLOGY PRADEEP VAIZ

turnitin | 22% SIMILAR | -- OUT OF 0

INTRODUCTION

"Insulin is not a cure for diabetes; it is a treatment. It enables the diabetic to burn sufficient carbohydrates, so that proteins and fats may be added to the diet in sufficient quantities to provide energy for the economic burdens of life" – **Sir Frederick Grant Banting.**

In present life situations, the incidence of diabetes mellitus is growing due to increase in population size, senility, modernization, obesity and decrease in exercise. The terms insulin-dependent and non-insulin-dependent diabetes have become obsolete to appear in the classification because these terms are often misused, based on the type of treatment the patient were receiving rather than their actual type of diabetes.

In the present scenario, type 2 Diabetes Mellitus occurs more frequently than other forms of diabetes. More often the predominant feature is decrease in the ability of insulin to act on tissues. Subjects 39 with type 2 DM usually have insulin resistance and is not frequently diagnosed for many years. Hyperglycemia develops slowly over years and so the typical features of diabetes mellitus are masked. Their circulating insulin levels may be normal or even higher.

Match Overview

66	Submitted to Universit... Student paper	<1%
67	Submitted to The Univ... Student paper	<1%
68	Asada, T.. "Overexpre... Publication	<1%
69	www.slideshare.net Internet source	<1%
70	Subramoniam, Appian.... Publication	<1%
71	Udo A. Zifko. "Somato... Publication	<1%
72	Submitted to Universit... Student paper	<1%
73	Textbook of Clinical Pe... Publication	<1%

PAGE: 2 OF 82 | Text-Only Report

Search the web and Windows | 16:07 14-09-2016

ACKNOWLEDGEMENT

First, I thank God the Almighty for providing me this opportunity to do a study and complete it successfully.

- I sincerely express my heartful gratitude to our beloved **Dean, Prof. Dr. Sithy Athiya Munavarah M.D.** and to our respected **Vice Principal Prof. Dr.S.M. Kannan M.S., Mch.,** Tirunelveli Medical College, Tirunelveli for their encouragement during the study period.
- I thank **Dr. Ratna Manjushree Jayaraman M.D. DCH,** Associate Professor and Head of the Department of Physiology for her tremendous support and valuable guidance during the study period.
- I take this opportunity to express my profound gratitude and sincere respect to my guide **Dr. B. Sujatha M.D., D.A.,** Associate Professor, Department of Physiology, Tirunelveli Medical College, for she has not only been my guide but a mentor who constantly wishes to support me any time during these three years.
- I am greatly indebted to **Dr. R. Saravanan MD., D.M.(Neurology),** Professor and Head, Department of Neurology for his expert guidance in midst of enormous workload.
- I thank Dr. R. Thenmozhi M.D. DCP, Associate Professor in Physiology and Dr. A. Jaya Jancy Selvi Ratnam M.D., DGO., Associate Professor in

Physiology for their constant support and valuable guidance in completing this study.

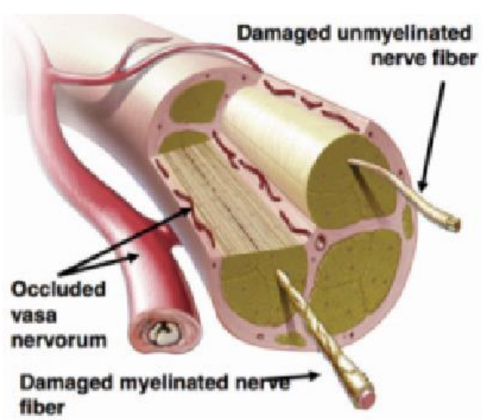
- I thank the Head of the Department of Medicine and Biochemistry, Tirunelveli Medical College for providing the subjects and laboratory respectively for the successful completion of the study. I thank all the lab technicians, Central Lab, Tirunelveli medical college hospital and Neurology lab technician for their support throughout my study.
- I thank the librarian, Mrs. M. Mala Shunmugapriya and all other staff of central library and my statistician, Mr. R. Jeromia Muthuraj for their loving support during the collection of reference articles.
- I am highly obliged to **Dr. N. Parvatharani**, Assistant Professor in Physiology and all other Assistant Professors and Tutors, in our department for their encouragement, and comments during the research period. My special thanks are **to my postgraduate colleagues,** for they gave me their helping hands when needed throughout the study.
- Last, but **an important note of thanks to all participants of this study** without whom this could not be accomplished.
- Above all, this study would be incomplete if my **wife and children** did not give the space I needed throughout the study period. **Heartful thanks and hugs to my family.**

CONTENTS

ABBREVIATIONS

AMP	Amplitude
ATP	Adenosine Tri Phosphate
BMI	Body Mass Index
Ca_v	Voltage-gated Calcium channel
CMAP	Compound Motor Action Potential
CV	Conduction Velocity
DM	Diabetes Mellitus
FFA	Free Fatty Acid
GLUT	Glucose Transporter
HbA1c	Glycosylated Hemoglobin
IGT	Impaired Glucose Tolerance
IAPP	Islet Amyloid Polypeptide
IGF	Insulin-like Growth Factor
KCNJ11	Potassium voltage gated channel subfamily J member 11
K_{ir}	Potassium inward rectifying channel
LAT	Latency
PI3K	Phosphotidyl Inositol – 3 Kinase
SNAP	Sensory Nerve Action Potential
SUR	Sulfonyl Urea Receptor

A STUDY TO CORRELATE SERUM MAGNESIUM, SERUM CALCIUM, HbA1c AND NERVE CONDUCTION PARAMETERS IN TYPE 2 DIABETES MELLITUS

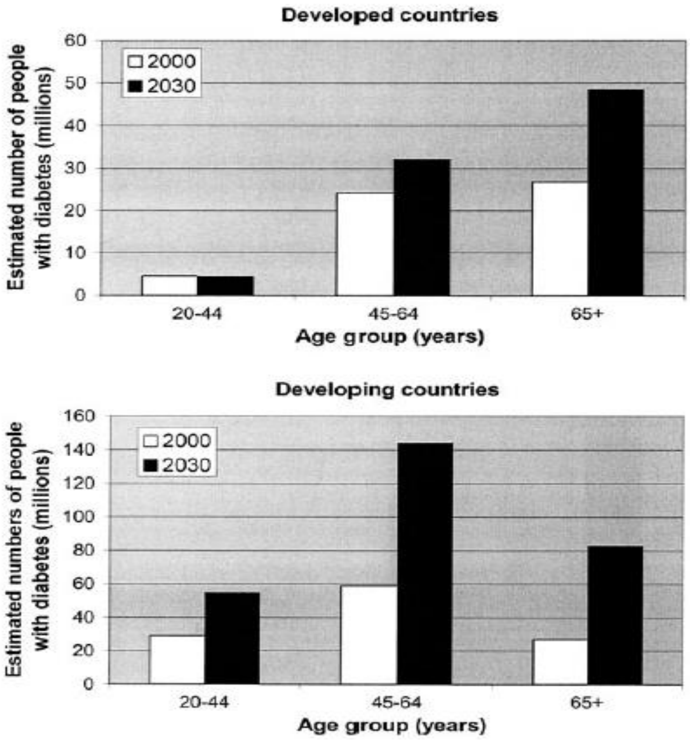


INTRODUCTION

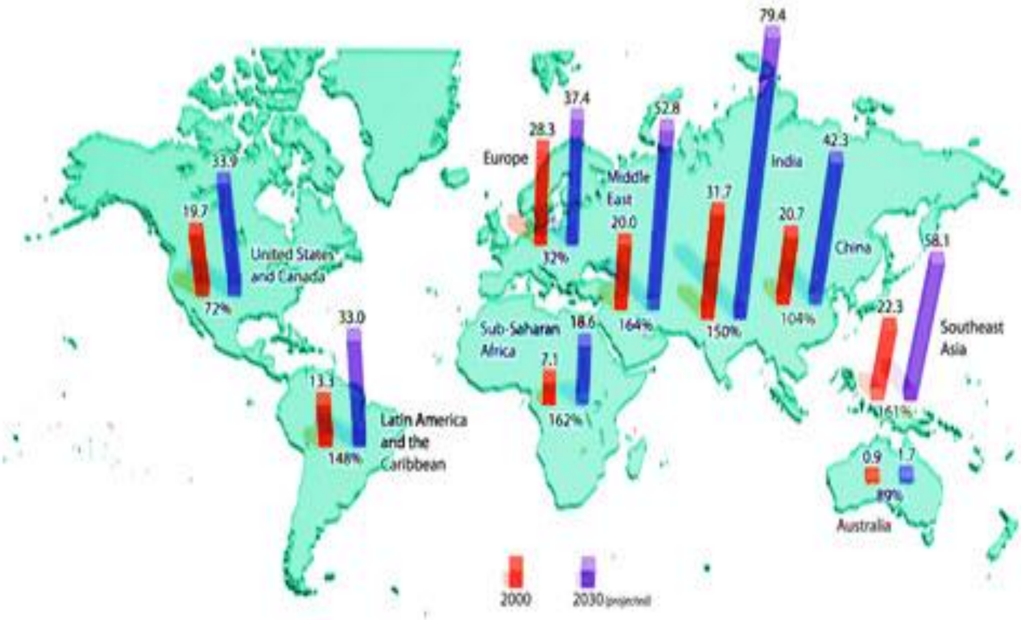
"Insulin is not a cure for diabetes; it is a treatment. It enables the diabetic to burn sufficient carbohydrates, so that proteins and fats may be added to the diet in sufficient quantities to provide energy for the economic burdens of life" – **Sir Frederick Grant Banting.**

In present life situations, the incidence of diabetes mellitus is growing due to increase in population size, senility, modernization, obesity and decrease in exercise. The terms insulin-dependent and non-insulin-dependent diabetes have become obsolete to appear in the classification because these terms are often misused, based on the type of treatment the patient were receiving rather than their actual type of diabetes.

In the present scenario, type 2 Diabetes Mellitus occurs more frequently than other forms of diabetes. More often the predominant feature is decrease in the ability of insulin to act on tissues. Subjects with type 2 DM usually have insulin resistance and is not frequently diagnosed for many years. Hyperglycemia develops slowly over years and so the typical features of diabetes mellitus are masked. Their circulating insulin levels may be normal or even higher.



Estimated No. of people with diabetes in developed & developing countries



Future percentage increase of diabetic population in different countries

Burden of disease:

In 2014, an estimated 422 million people were affected with diabetes throughout the world. This estimate was only 108 million in 1980. The prevalence throughout the world in 1980 was 4.7%. The prevalence in 2014 has increased to 8.5%. This increase in prevalence was accounted for an increase in life style modifications and obesity. After the year 2000, people living with diabetes have risen considerably in developing countries than in developed countries[1].

In 2012, Diabetes mellitus caused 1.5 million deaths. Hyperglycemia per se by increasing the risks of vascular and other organ morbidities caused an additional 2.2 million deaths. So, totally it accounted for 3.7 million deaths out of which 43.3% were less than 70 years of age. The death percentage due to hyperglycemia in age groups less than 70 years is more in developing countries than in developed countries. The laboratory tests that are required to distinguish between type 1 and type 2 DM are costly and so, separate global estimates of prevalence of two types of diabetes mellitus do not exist. Type 2 diabetes mellitus is often a disorder that occurs in adults. Now it’s incidence in children is increasing.

The International Federation for diabetes projects that 438 million individuals will be affected by this disorder by the year 2030. Although the prevalence of both type 1 and type 2 DM is increasing worldwide, the prevalence of type 2 DM is raising much more rapidly. The cause for this rapid increase would be increase in percentage of obese population, decrease in physical activity of the population and industrialization. The prevalence is alike in men and women above 20 years of age (11.8% and 10.8%, respectively). The estimates revealed that in 2030, the greatest number of individuals with type 2 diabetes mellitus will be in the age between 45 and 64. The most of the affected population are from developing countries.

The epidemiological distribution of diabetes worldwide is projected for 2030 in which our country stands first. The projected increase of diabetes population in the year 2030 for our country is 150%.

Diabetes mellitus, showing an iceberg phenomenon cause a significant economic burden to the global health-care system. This burden can be direct or indirect. Direct medical costs incurred are used for preventing and treating diabetes and its related complications. The costs incurred included emergency care, out-patient care, in-patient care, supply of medicines and consumables. The current IDF estimates project money spent on diabetes mellitus is three times more than compared to previous ten years. The major contributing factor for the rise in expenditure was purchase of patented and branded medicines to treat the patients. Developing countries carry a larger proportion of this future global health-care expenditure burden than developed countries. The indirect burden is posed on the families of affected individual. There is loss of family income associated with disability of the individual or early death of the individual. A research says that diabetic population has a chance of spending huge amount of money on illnesses when compared to the population without DM. These effects were significant in developing countries[2].

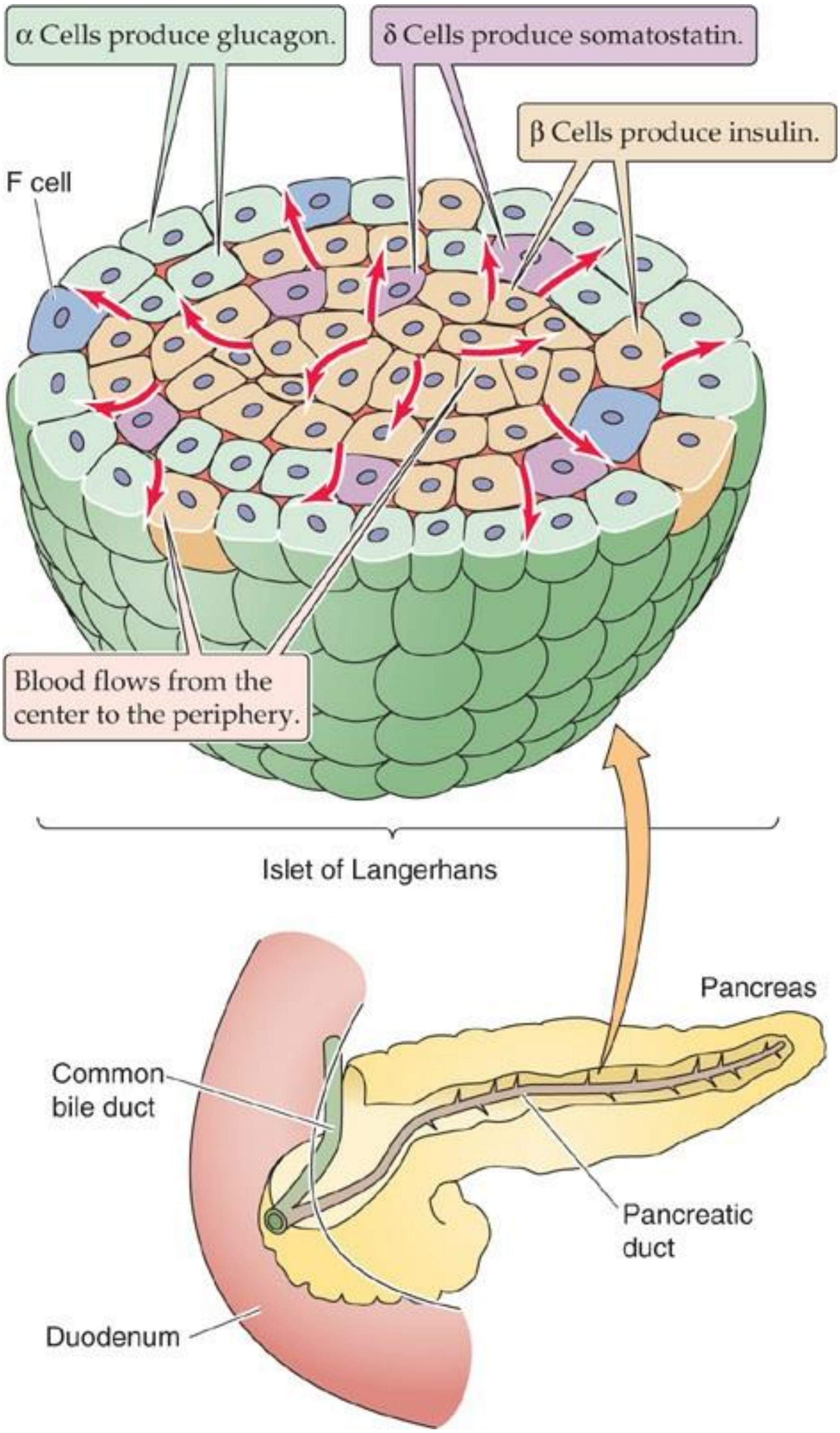
AIM AND OBJECTIVES

1. To compare HbA1c, Serum magnesium and serum calcium between newly diagnosed type 2 DM and type 2DM with duration of more than five years.
2. To compare the nerve conduction parameters of individual nerves between newly diagnosed type 2 DM and type 2DM with duration of more than five years.
3. To correlate HbA1c with nerve conduction parameters of individual nerves and compare between the two groups.
4. To correlate HbA1c with serum magnesium and body mass index and compare between the two groups.
5. To correlate serum magnesium with nerve conduction parameters of individual nerves in two groups.

REVIEW OF LITERATURE

Introduction to Endocrine Pancreas:

Pancreas is a composite lobulated organ. It has exocrine and endocrine actions. It resembles upper end of a thick walking stick lying sideways with handle on right and turned downwards. It develops from two endodermal buds namely dorsal and ventral that arise from the part of the gut that later becomes the second part of duodenum. The secretory components are derived from the proliferation of the primitive ducts. The islets of Langerhans are derived from the primitive duct system[3]. More than one million islets are found in human pancreas. An islet consists of polygonal or round cells arranged in cords and separated by blood capillaries. There is a slight abundancy of islets in the tail of pancreas. Immunocytochemical methods identify four types of cells –A, B, D and F. In humans A-cells have regular granules with a dense core surrounded by a clear region bounded by membrane. They contain immune reactive glucagon. A-cells are identical with those found in the gastric antrum. B-cells have irregular granules that are rhomboid shaped with a core formed of irregular crystals of insulin in complex with zinc. Both endocrine cells and blood vessels of islets are innervated by autonomic nerve fibres. A-cells are predominant in periphery. B-cells are usually concentrated in central region. The distribution of D and F cells are variable. D-cells contain granules of low density and immunoflurosence studies suggest the presence of gastrin. A-cells and B-cells are separated only by peri-capillary spaces, so glucagon could easily be transported to act directly on B-cells and thus promote the secretion of insulin[4].


α Cells produce glucagon.
δ Cells produce somatostatin.
β Cells produce insulin.
F cell
Blood flows from the center to the periphery.
Islet of Langerhans
Pancreas
Common bile duct
Pancreatic duct
Duodenum

Islet of Langerhans

PATH TO INSULIN

This story of earliest identification of diabetes mellitus and the way to the discovery of the remedy is filled with triumphs, defeats and serendipity.

- The best earliest evidence occurred in 1550 B.C. when a symptom of diabetes mellitus was recorded in Ebers papyrus. Then the description of polyuria to Imhotep, a man of medicine, architecture and magic, who was a high priest and minister to the Pharoah Zosser in 3000 B.C. was documented.
- Galen and Arateus, the two Greek physicians described the disease further. Arateus in his work "Acute and Chronic disease" coined the term "Diabetes" meaning "Siphon" to explain "the liquefaction of the flesh and bones into urine".
- Susruta, an ancient Indian surgeon in 400 B.C. has described the diabetic syndrome as "Honeyed urine".
- In 1674, Thomas Willis, a physician, an anatomist and professor of natural philosophy in Oxford university identified that individuals with diabetes produced sweet urine. He discovered that by "Tasting".
- In 1776, Mathew Dobson from Manchester demonstrated that the persons with diabetes mellitus actually excrete sugar in urine.
- In 1815, French chemist Cherreul discovered that the sugar in diabetic urine was glucose.
- In 1850, Claude Bernard described the 'internal secretion' of glucose into blood from its storage form 'glycogen' in the liver.
- The pancreatic islets were discovered in 1868 by Paul Langerhans when he was a medical student in Berlin.

- Minkowski and Von Mering in 1889 demonstrated that pancreatectomy from dogs caused increase in plasma glucose, increase in urination, increase in thirst, weight loss and finally death, a syndrome closely resembling type 1 DM. Following this lead, a group of investigators in the Department of Physiology at the University of Toronto prepared extracts of pancreas and tested the ability of these extracts to lower plasma glucose in pancreatectomized dogs. Despite months of failures, these investigators persisted in their belief that such extracts could be beneficial.

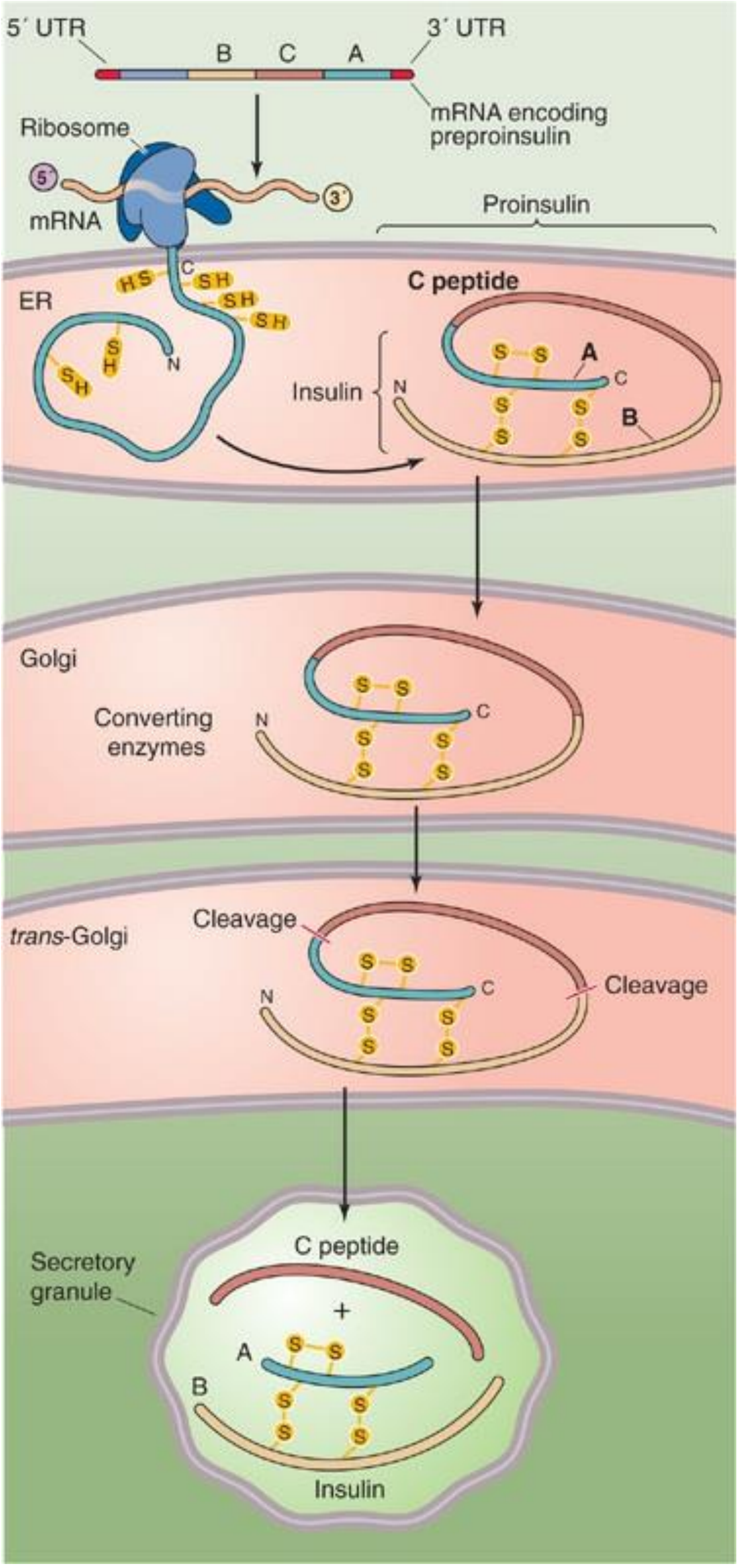
- It was in 1921, when Sir Frederick Grant Banting-a young surgeon;John Macleod-Professor of physiology;Charles Herbert Best-a fourth year medical graduate as well as an assistant to John Macleod and J.B. Collip a skilled chemist commenced their work in the laboratory of Prof. Mcleod. They did their experiments on dogs and isolated a substance, ‘Isletin’ now called insulin which found to decrease blood glucose level. Nobel Prize was awarded in October 1923, 18 months after their discovery. That event was a gripping tale of success, disappointment and conflict. Banting and Macleod were honoured, but still both shared their prizes with the counterparts. The first patient to be treated with the extract of the substance was Leonard Thompson 14 year old boy with metabolic acidosis[5].
- In 1926, crystalline insulin was prepared by Abel. Sanger and coworkers, in 1955 delineated the structure of insulin. Proinsulin was discovered in 1967.
- First pancreas transplant was conducted in 1967. Lacy and coworkers started islet cell transplants in the year 1989[6].

Synthesis of Insulin:

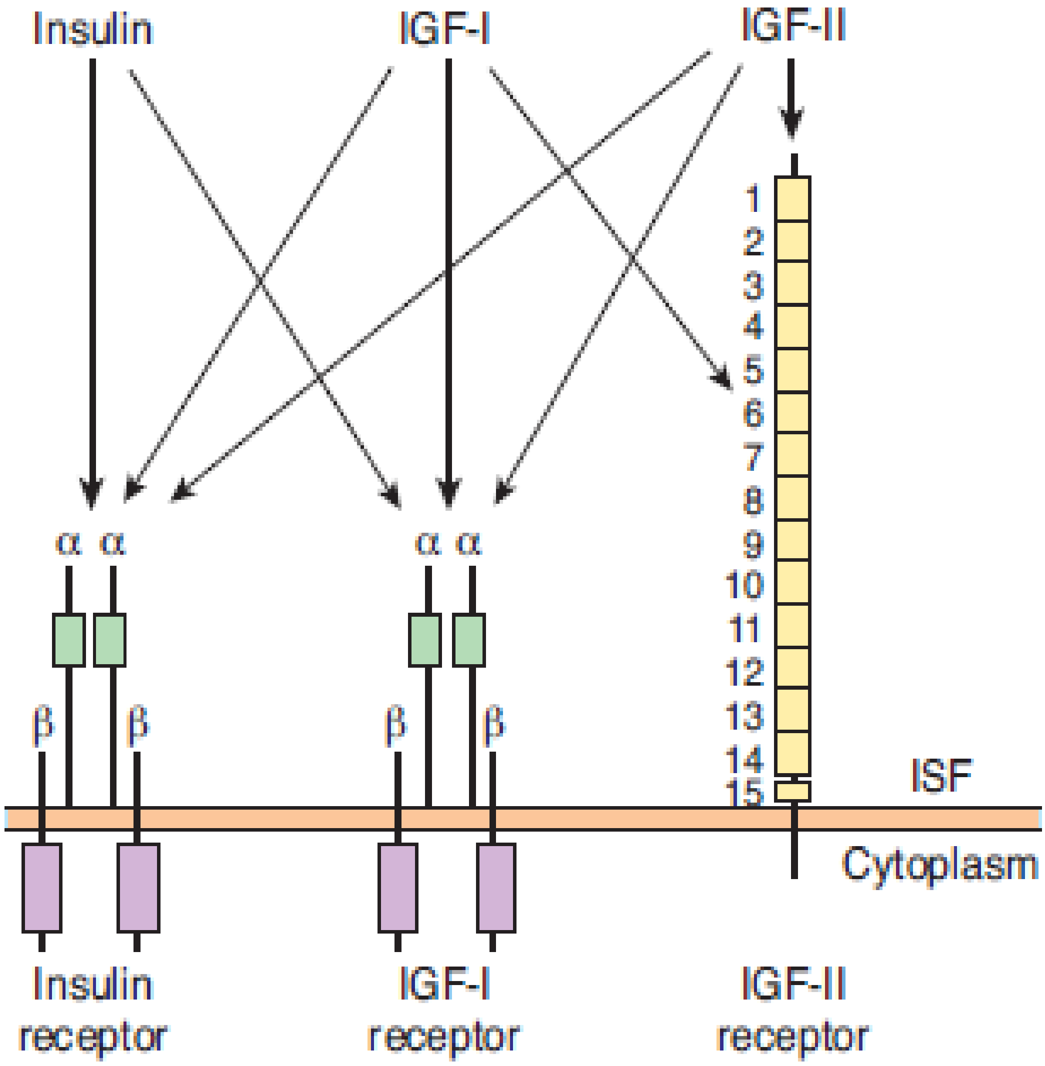
In humans, the gene for insulin is located on the short arm of chromosome 11. Insulin as any other protein is produced in the rough endoplasmic reticulum of beta cells of pancreas. It is then taken to the Golgi apparatus for packaging and these packed substances move to the beta cell membrane and is exocytosed. Insulin is produced as part of a larger pre-prohormone which after cleavage of a peptide forms pro insulin. The connecting peptide (C peptide), connects A and B chains and facilitates the folding after which it is detached in the granules before secretion. The insulin then enters the neighboring fenestrated capillary endothelium to reach the bloodstream. Insulin constitutes about 90–97% of the products released from the β cells. The level of C-peptide which is secreted in equimolar concentrations of insulin is determined by radio immunoassay. The C-peptide level in blood serves as a marker of β cell function in patients receiving parenteral insulin. The half-life of insulin in the circulation in humans is 5 minutes. Insulin binds to its receptors found on many different cells in the body. It also binds to cells in which they do not increase glucose uptake[7].

Insulin Receptor:

The gene for receptor of insulin is located on chromosome 19. It has a molecular weight of approximately 340,000. It is a tetramer made up of 2 alpha and 2 beta glycoprotein subunits synthesized by a single mRNA and



Synthesis and processing of insulin molecule



Insulin, IGF-1 and IGF-II receptors

then separated by proteases. They are bonded to each other by disulfide bonds. The alpha subunits bind to insulin molecules and are present extra-cellularly. The β subunits span the cell membrane. The intracellular portions of the β subunits have tyrosine kinase activity. Both subunits are glycosylated. When insulin binds to the receptor, the tyrosine kinase activity of the β subunits, produces autophosphorylation of the β subunits.

Mice knocked out of insulin receptor gene show features of growth stunting in fetal period. They have abnormalities of skin, nervous system, and die at birth due to respiratory insufficiency. The protein anabolic effects of insulin are mediated through PI3K. This enzyme pathway is involved in the growth of nerve cells in the visual system. The insulin receptor is very similar to IGF I receptor but different from IGF-II receptor. The growth factor receptors also have tyrosine kinases but their amino acid composition is different. When insulin binds to its receptors, they form an aggregation and then these patches are taken into the cell by receptor mediated endocytosis. The insulin–receptor complexes enter lysosomes, where the receptors are broken down or recycled. The half-life of the insulin receptor is around 7 hours[8, 9].

Actions of Insulin[10,11]:

TIME OF ONSET	ACTION
RAPID-in seconds	Increased transport of glucose into insulin sensitive cells
	Increase in transport of amino acids into cells sensitive for insulin
	Increased transport of K^+ into insulin sensitive cells
INTERMEDIATE -in minutes	Protein synthesis stimulation
	Protein degradation inhibition
	Activation of enzymes of glycolysis and glycogen synthetase enzyme
	Inhibition of enzymes such as phosphorylase and few gluconeogenic enzymes
DELAYED-in hours	mRNAs for lipogenesis increases

Effect of Insulin in Various Tissues:

General:

Growth of all cells is increased

Adipose tissue:

Increases entry of glucose into cells

Increases synthesis of fatty acids

Increases the production of glycerol phosphate

Activates lipoprotein lipase

Increases potassium uptake

Increases the deposition of triglycerides

Muscle:

Increases synthesis of glycogen

Increases entry of glucose

Amino acid uptake is increased

Ribosomal protein synthesis is increased

Protein breakdown is decreased

Decreased release of gluconeogenic amino acids

Increased ketone uptake

Potassium uptake is increased

Liver:

Ketogenesis is decreased

Protein synthesis is increased

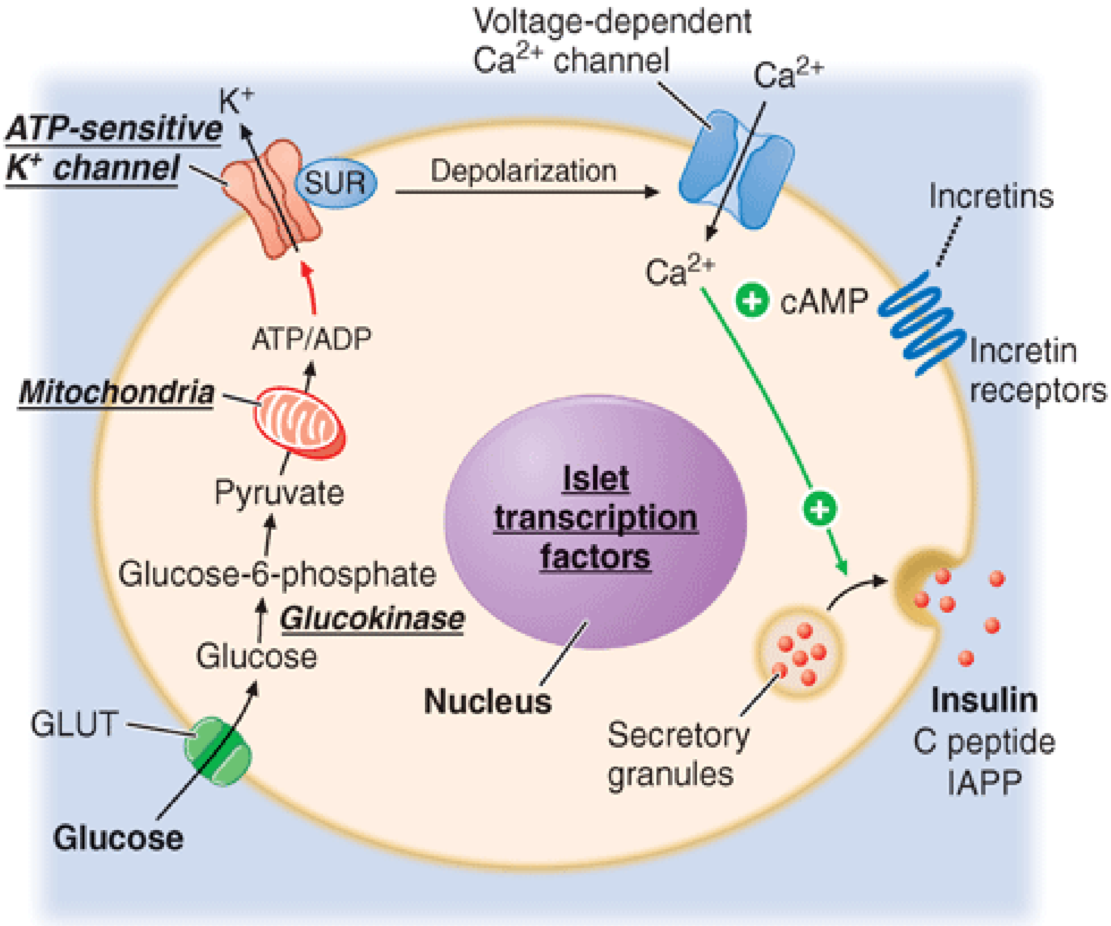
Lipid synthesis is increased

Glucose output is decreased due to decreased gluconeogenesis

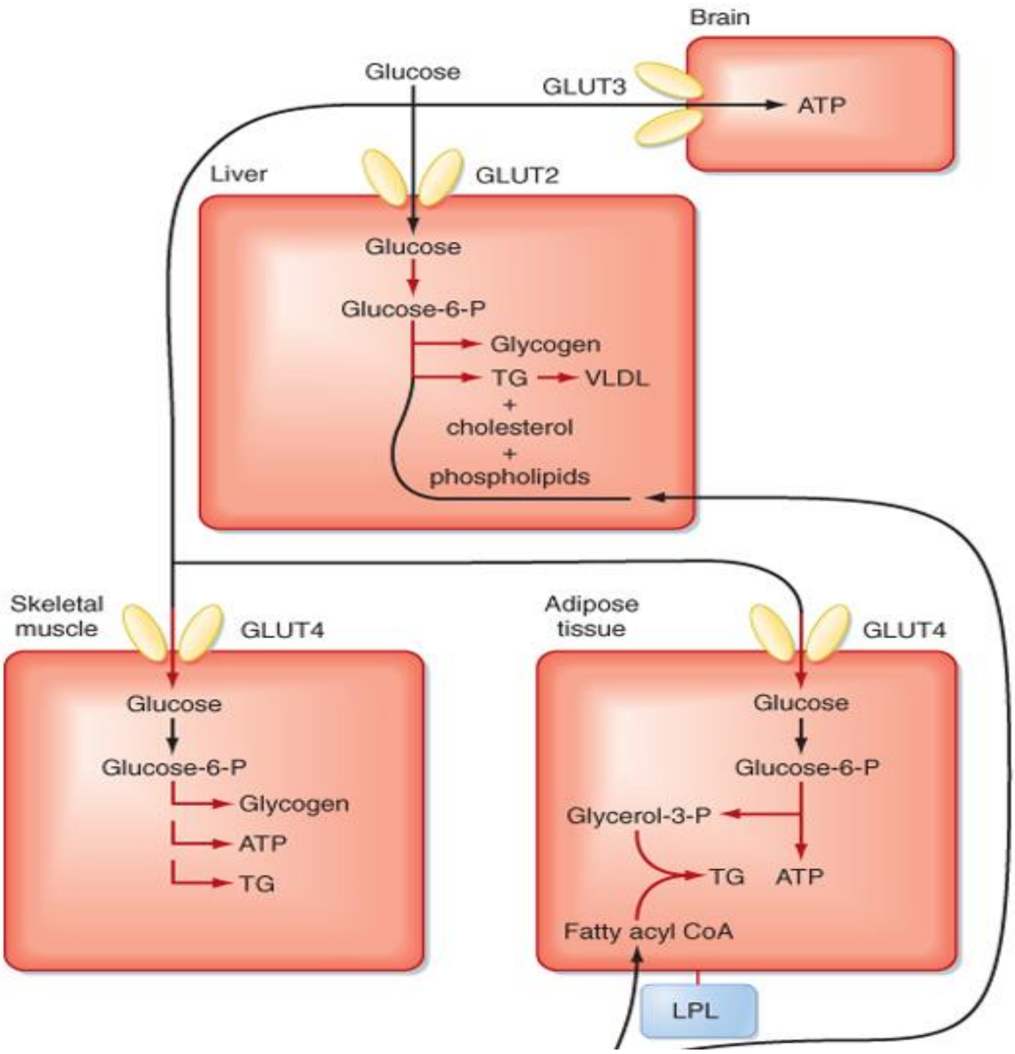
Glycolysis and glycogen synthesis are increased[12]

Regulation of Insulin Secretion:

Insulin secretion is chiefly regulated by blood glucose levels through negative feedback. On entry into the cell glucose is metabolized to ATP. This accumulation of ATP causes closure of potassium channels and result in cell depolarization. This depolarization cause Ca^{2+} influx activates kinases and thus resulting in insulin secretion.



Glucose mediated insulin secretion



Glucose transporters and Glucose utilization in various tissues

Amino acids, especially arginine, leucine and lysine increases insulin secretion. The secretion of insulin is increased after a protein meal. Arginine is a precursor of nitric oxide and so, nitric oxide itself stimulates insulin secretion. Fatty acids and ketone bodies increase insulin secretion when their blood levels are high.

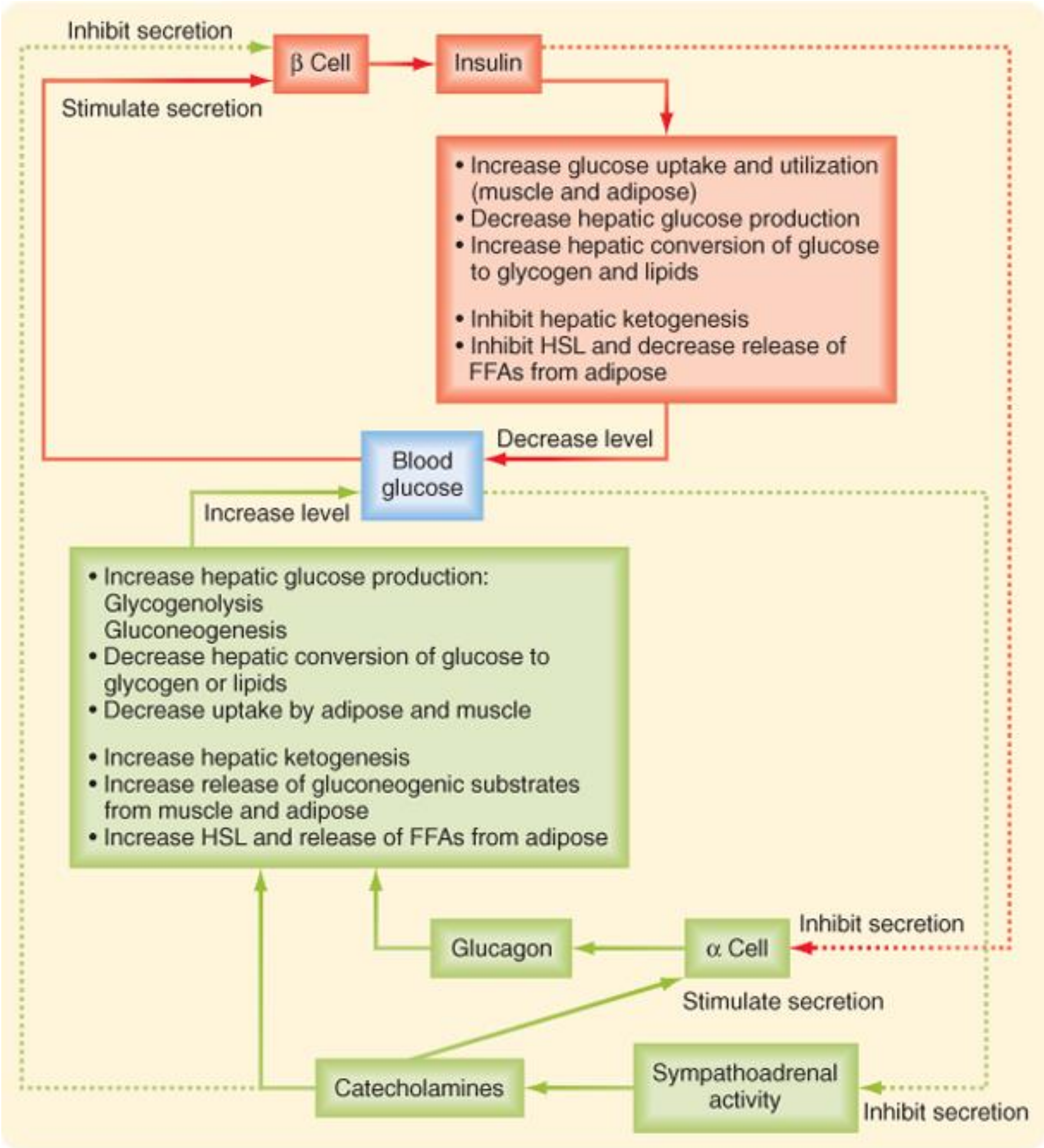
Influence of gastrointestinal hormones- Gastric inhibitory peptide, gastrin, secretin and cholecystokinin-pancreozymin increases insulin secretion. The physiological “gut factor” gastric inhibitory peptide is found to be the cause of increased insulin secretion following an orally administered glucose load than a same dose administered intravenously. Acetylcholine increases insulin secretion whereas it is blocked by atropine. β-adrenergic blockers and α-adrenergic agonists inhibit insulin secretion whereas β- adrenergic agonists increase insulin secretion[13].

Glucose Metabolism:

Glucose stimulates insulin secretion when blood glucose concentration is over 70 mg/dl. Glucose is transported into beta cells by facilitated diffusion through a transporter protein, called GLUT. The rate-limiting step in glycolysis is the phosphorylation of glucose by glucokinase that controls glucose-regulated insulin secretion. This metabolism of glucose through glycolysis generates ATP, which accumulate and inhibit the activity of an ATP-sensitive K^+ channel. This ATP-sensitive K^+ channel consists of a binding protein site for certain oral hypoglycemic, and the other protein site is an inwardly rectifying K^+ channel protein. Inhibition of this potassium channel induces beta cell membrane depolarization, which opens voltage-dependent calcium channels. This calcium released by the above process cause insulin secretion from the stored granules. Insulin is secreted in a pulsatile pattern, with small secretory bursts occurring every 10 minutes. Incretins are produced in the neuro-endocrine cells of the gastrointestinal tract. These are produced following food ingestion and they amplify glucose-stimulated insulin secretion and suppress glucagon secretion. Glucagon-like peptide-1 is the most potent incretin. It is released from 'L' cells in the small intestine and it stimulates secretion of insulin only when the blood glucose is above the fasting level[14].

Glucose homeostasis:

Homeostasis is maintenance of constancy. Glucose is maintained in a constant concentration when there is a balance between production of glucose and its peripheral uptake and utilization. Neural signals, metabolic inputs, and various hormones result in an integrated control of glucose supply and utilization of glucose. Among all, the prime regulator is insulin. In the fasting state, low insulin levels promotes gluconeogenesis and glycogenolysis in liver and reduce glucose uptake in insulin-sensitive tissues like skeletal muscle and adipose tissue. This promotes the mobilization of stored precursors such as amino acids and free fatty acids. Glucagon is



Feedback loops of insulin secretion

another hormone produced by alpha cells of pancreas. It stimulates gluconeogenesis and glycogenolysis when insulin is low. Postprandially, the glucose load cause a rise in insulin and fall in glucagon, leading to a reversal of these processes. Insulin is an anabolic hormone. It promotes the storage of carbohydrate and fat and helps in protein synthesis. The majority of postprandial glucose is utilized by skeletal muscle, by a mechanism called insulin-stimulated glucose uptake. Other tissues, most notably the brain, utilize glucose in an insulin-independent fashion[15, 16].

NORMAL NERVE METABOLISM AND PHYSIOLOGY:

Mammalian tissues, under physiological conditions maintain a dynamic balance between the rate of production and utilization of ATP. Whenever there is a restriction of ATP production, the cells use their phosphocreatinine stores for ATP production, decrease the energy requiring biological activities or increase glycolysis for ATP production. Under physiological conditions, glucose is the major substrate for energy production in the peripheral nerve endoneurium. Insulin does not regulate the metabolism of these tissues. Cells cannot survive for prolonged periods of restricted ATP production, particularly if it is due to ischemia. In ischemia there is reduction of not only oxygen but also provision of adequate glucose required for glycolysis. This results in rapid accumulation of lactate and development of acidosis.

PHYSIOLOGY OF NERVE CONDUCTION:

Historical Aspects:

- Invention of cathode ray oscilloscope in 1897 by Braun was the breakthrough in the study of action potentials.
- In 1903 string galvanometer was found out by Einthoven. Muscle action potential instead of the muscle twitch was recorded in the measurement of conduction velocity in motor nerves by Piper in year 1909 and Munnich in 1916.
- By stimulating tibial nerve Hoffmann demonstrated the monosynaptic reflex in soleus muscle. It was named as H reflex in 1918.
- In 1922, Joseph Erlanger and Herbert Spencer Gasser, a student of the former found out that difference in conduction velocity of in nerve fibres is directly proportional to diameter of nerve fibres. Based on this finding they grouped nerve fibres into three different groups. The large nerve fibres were grouped as type A-fiber with maximum velocity and smaller fibres as type C with minimum velocity. In 1944 they received Nobel Prize for the same[17].
- By stimulating the motor nerves Harvey and Masland observed the decremental response in myasthenia gravis in 1941. In 1957 Eaton and Lambert used the same procedure in various neuromuscular disorders including myasthenic syndrome.

- Alan Lloyd Hodgkin and Andrew F. Huxley did study on Squid and cattle fish having giant axons because it is easy to introduce many electrodes and measure the resting membrane and action potential. They demonstrated the membrane permeability to sodium and potassium in various phases of action potential and also refractoriness of nerve fibres for about 1 ms. They were the pioneers in devising voltage clamp method. They also found out the different bands in skeletal muscle fiber and elaborated the "sliding filament theory" of muscle contraction. In 1963, Hodgkin, Huxley and John Eccles were awarded the Nobel Prize.
- Changes in the nerve conduction parameters in peripheral neuropathies were studied by Harvey and Kutfer by doing nerve conduction study. The slowing effect of nerve impulse propagation in ischemia was the observation by Kugelberg in 1944 and Cobb and Marshall in 1954[18]. Conduction velocity was calculated for the first time by Hodes, Laravee and German in 1948.They did it so by stimulating a nerve at its various levels. The principle of photographic superimposition in the calculation of nerve conduction velocity in sensory nerve was used by Dawson and Scott in 1949[19]. Dawson by devising digital nerve stimulation technique in 1956 differentiated the sensory potential from antidromic impulse in motor nerve. Use of

nerve conduction studies in the differentiation of demyelinating disease and axonal neuropathy was adopted by Lambert and Kaeser.

- From the year 1960, sensory nerve conduction study was made as an integral part of electro diagnostic study in neurophysiology.

MOTOR CONDUCTION STUDY:

In motor conduction study, nerve is stimulated over two points with supra threshold strength. Minimum distance of 10 cms is required between two points of stimulation. This distance is changed to shorter segments in the evaluation of entrapment neuropathies like carpal tunnel syndrome. The latency in milliseconds is taken from the point of stimulus-artifact to the first negative deflection. Onset latency includes residual latency also. Residual latency is constituted by neuromuscular transmission time and propagation time along the muscle membrane. For amplitude measurements, either baseline to the first negative peak or from a peak to the next peak is used. The unit of amplitude is the millivolt. Duration is measured between onset of negative peak or onset of positive peak or onset to final return to baseline. The conduction velocity in meter per second is calculated by dividing the distance between two points and the difference between the two latencies. Elimination of residual latency is thus achieved by measuring the difference between the two latencies. Latency denotes the conduction of the impulse in fast conducting motor fibres and number of intact nerve fiber is indicated by the amplitude. There is a correlation between the density of small fibres and the duration of the amplitude[20].

F Wave:

When motor neurons are stimulated antidromically, it results in conduction of the impulse to and from spinal cord. It is done by stimulus with supra maximal strength. The cathode is placed proximally and anode distally.

SENSORY CONDUCTION STUDY:

There are two types of sensory conduction measurements. In antidromic conduction study, action potential is recorded over the distal point on the nerve while the stimulation is applied on the proximal point of the nerve. Reverse process is followed in orthodromic conduction. Distal portion is usually a digital nerve. The surface and ring stimulating electrodes are used for antidromic and orthodromic conduction measurement respectively. Needle electrodes are used in difficult situations.

SNAP is a triphasic waveform in orthodromic conduction. Triphasic response is due to initial positive peak. This initial positive peak is absent in antidromic conduction studies. Sensory latency is a measurement between the stimulus-artifact to the first positive peak or negative peak. The amplitude of SNAP is either measured between the base line to negative peak or between negative and positive peaks. Measurement between initial positive peak and the intersection of descending phase to base line gives a measure of duration of SNAP. It can also be measured between the initial positive peaks to final return to baseline or between initial positive peaks to subsequent positive peak. As no residual latency is present in sensory conduction study, nerve conduction velocity can be determined by stimulating the nerve at only one point. Distance between stimulating and recording site divided by latency gives rise to velocity of sensory nerve fibres. The duration signifies the number of fibres of slow conducting type and the amplitude indicates the density of nerve fibres[21].

FACTORS INFLUENCING CONDUCTION PARAMETERS:

These factors are due to physiological variation or the technical factors involved in conduction study. Age, limb under study and body temperature are the physiological variables affecting the conduction studies .Technical factors influencing are the stimulating and recording systems of the equipments used and faulty stimulation of the nerve not under study and abnormalities of the nerves.

(i) AGE:

Myelination is not complete at birth. It takes two years for completion of myelination after birth. So in a new born child or infant, the conduction velocity is low as compared to adults. It reaches the normal value of the adult around three to five years of age. Even though degenerative changes in nerves take place in old age, the conduction velocity is not decreased more than 10 meters per second even at eighty years of age. There is a positive correlation between latency of F wave and advancing age[22].

(ii) LIMB UNDER STUDY:

Nerves of the upper limbs conduct the nerve impulse faster than the nerves of the lower limb. It applies to both motor and sensory nerves. This is due to the fact that length of the upper limb nerves is shorter than that of lower limb nerves. Temperature of the hands is more than the temperature of feet. Proximal nerve in a limb conducts impulse faster than the distal nerves.

(iii) TEMPERATURE:

The change in the temperature affects the nerve conduction parameter in both motor and sensory conduction studies. If the temperature decreases by one degree Celsius, there is an increase in latency of 0.3 meter per second. Change in the temperature alters the activity of sodium channels. When the temperature is changed from 29 degree celsius to 38 degree Celsius, the conduction velocity is increased by 5 % per degree celsius. This error can be avoided by performing the study in room temperature 21 to 23 celsius. If measured limb temperature is below the 34 degree Celsius the warming of limb is to be carried out by immersing the limb in hot water or by using infrared lamps[23].

(iv) STIMULATING SYSTEMS:

Failure in stimulating system leads to sub maximal stimulation of nerves. Due to the failure, the applied stimulus fails to travel up to the target part. Both these factors bring about no response or reduced response in the study. The failure may be due to the incorrect placement of stimulators. Sweat may influence the conduction. In order to avoid the failure of the system, the stimulating electrodes should be placed closure to the nerve and with the adequate firm pressure. If patient is obese or has edema needle electrodes can be used.

(v) RECORDING SYSTEM:

Incorrect placement of active or reference electrode alters the wave pattern. For example, if active electrode is placed in incorrect position, an initial positive wave is produced instead of initial negative wave. This can also occur due to the abnormalities of nerve supply or unintentional stimulation of nearby nerves. Hence an investigator should know the anatomical variations of the nerve.

(vi) INADVERENTLY STIMULATING THE NERVES NOT STUDIED:

The applied stimulus current sometime stimulates the nearby nerve or nerve roots which are not involved under study. This leads to false result in latency variables.

(vii) ABNORMALITIES IN THE INNERVATION PATTERN:

Abnormal connection between the nerve produce changes in the amplitude. These abnormal connections may be in the form of connection between the median nerve and ulnar nerve (Martin Gruber anastomosis). This abnormal connection leads to abnormal nerve supply. So before starting nerve conduction studies, all the above factors changing the result should be kept in mind to diagnose the nerve conduction abnormality[24].

PATHOPHYSIOLOGY OF DIABETES MELLITUS

Definition:

Diabetes mellitus is a heterogeneous group of metabolic disorder characterized by elevated blood glucose and associated with disturbances in carbohydrate, fat and protein metabolism resulting from defect in secretion of insulin, action of insulin action or both.

Criteria for Diagnosing Diabetes Mellitus:

- Symptoms of DM plus blood glucose taken randomly> 200 mg/dl
- Blood glucose taken in fasting > 126 mg/dl
- 2 hour blood glucose > 200 mg/dl after an oral glucose tolerance test[25]

Any one of the above criteria can be used for diagnosing DM.

Genetics of type 2 DM:

A powerful approach to the identification of genetic variants that predispose to type 2 DM is still on a hunt while a lot have already been explored. Single nucleotide polymorphism has created a path in identifying the possible variations involved in its genetics. Genetic studies were based on two strategies. First is to focus on the functional candidate genes and other on the positional candidate genes.

Studies of functional candidate genes involved genes responsible for the actions of insulin. Mutation in the insulin receptor gene, though uncommon have been implicated in severe form of insulin resistance in type 2 DM. One of the best characterized molecules in the intracellular insulin action cascade is insulin receptor substrate (IRS-1). This is a cytosolic protein that is tyrosine phosphorylated by the insulin receptor. A total of nine substitutions have been identified till now out of which the most prevalent is the glycine-to-arginine substitution. PI-3 kinase is the major protein that is bound and activated by phosphorylated IRS. A reduction in the insulin response of this enzymatic activity has been reported in muscle and adipose tissue from insulin-resistant subjects with type 2 DM. While insulin has pleiotropic effects on cellular functions, the actions that have been specifically shown to be impaired in type 2 DM are those related to glucose uptake and incorporation into glycogen. GLUT-4 gene and glycogen synthase gene have been extensively studied. Multiple defects in the activity of this enzyme, glycogen synthase in the skeletal muscle of patients with type 2 DM have been positively correlated. Genes involved in GLUT-2 and the enzyme glucokinase play a central role in sensing of glucose. Glucokinase mutations cause maturity onset diabetes of young. Adenine nucleotide-sensitive potassium channels play a vital role in coupling glucose metabolism to insulin release. The genes encoding for the subunits of this channel are called KCNJ11 & SUR1.

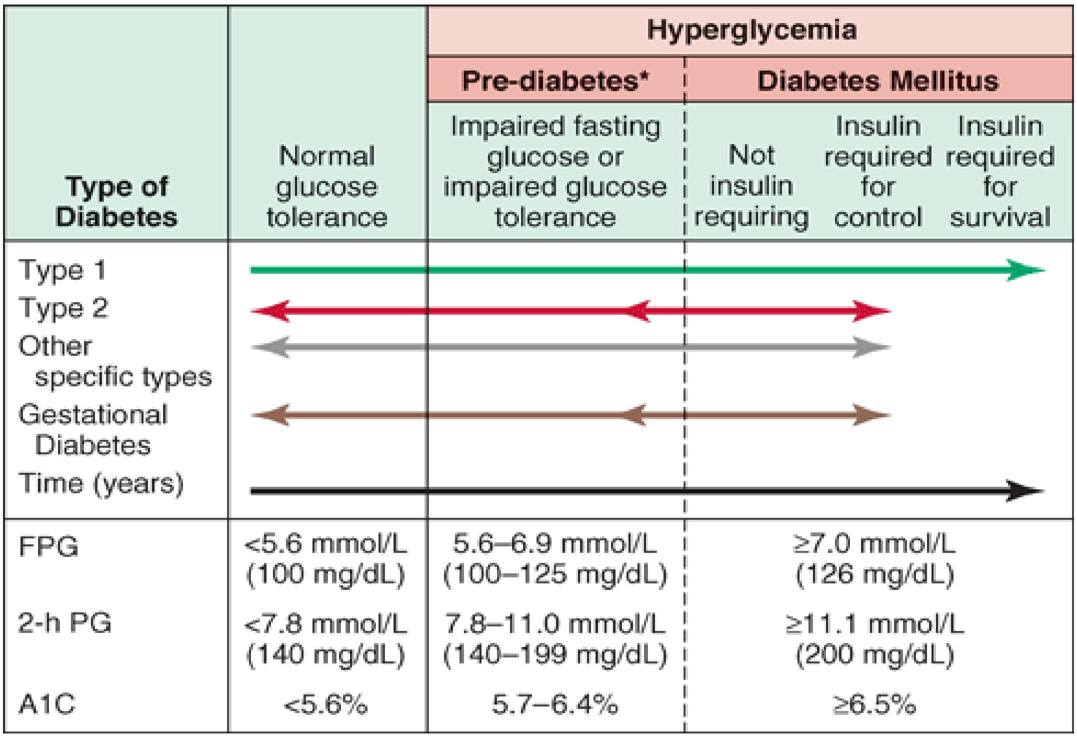
Diabetes mellitus was previously classified on the basis of the age of diagnosis or based on therapy but now it is classified based on the etiopathogenetic process that leads to persistent elevation in blood glucose.

The two broad types of DM are designated type 1 and type 2. Both types of diabetes have a phase of abnormal glucose homeostasis as the disorder progress. Type 1 DM is the result of complete or near-total deficiency of insulin.

Type 2 DM is a group of disorder characterized by variable degrees of insulin resistance, impaired insulin secretion, and increased glucose production. Type 2 DM is preceded by a period of abnormal glucose homeostasis classified as impaired fasting glucose (IFG) or impaired glucose tolerance (IGT). Visceral or central obesity is very common in type 2 DM. In the early stages of the disorder, pancreatic beta cells compensate by increasing insulin output and so glucose tolerance remains near-normal, despite insulin resistance.

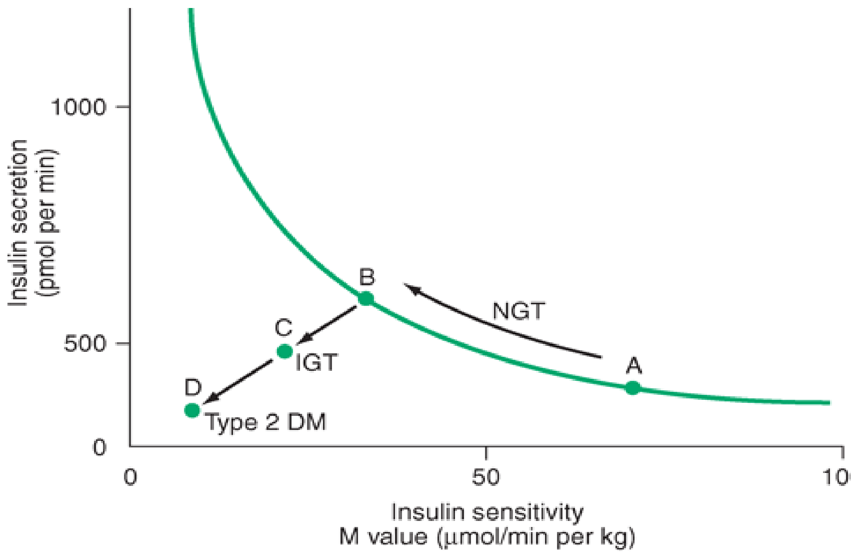
As insulin resistance and compensatory hyperinsulinemia advances, endocrine pancreatic cells are unable to sustain the hyperinsulinemic state. Impaired glucose tolerance, followed by elevations in postprandial blood glucose develops. A further decline in insulin secretion and an increase in hepatic glucose production lead to overt diabetes with fasting hyperglycemia. At last, pancreatic beta cell failure occurs.

Insulin secretion and insulin sensitivity are inter-related. When an individual becomes more insulin resistant or when insulin sensitivity decreases (curve moves from point A to point B), insulin secretion increases.



Type of Diabetes	Normal glucose tolerance	Hyperglycemia			
		Pre-diabetes*	Diabetes Mellitus		
		Impaired fasting glucose or impaired glucose tolerance	Not insulin requiring	Insulin required for control	Insulin required for survival
Type 1					
Type 2					
Other specific types					
Gestational Diabetes					
Time (years)					
FPG	<5.6 mmol/L (100 mg/dL)	5.6–6.9 mmol/L (100–125 mg/dL)	≥7.0 mmol/L (126 mg/dL)		
2-h PG	<7.8 mmol/L (140 mg/dL)	7.8–11.0 mmol/L (140–199 mg/dL)	≥11.1 mmol/L (200 mg/dL)		
A1C	<5.6%	5.7–6.4%	≥6.5%		

Spectrum of Glucose Homeostasis and Diabetes Mellitus



Relationship of insulin sensitivity and secretion in IGT and Type 2 DM

A failure to compensate by increasing the insulin secretion occurs and results in IGT (point C) and finally to type 2 DM (point D).

Insulin secretion and sensitivity are inter-related. In type 2 DM, insulin secretion initially increases in response to insulin resistance to maintain normal glucose levels. Initially, secretion of insulin is affected mildly and it particularly involves glucose-stimulated insulin secretion. The response to other substances, such as arginine, is preserved. The abnormalities in proinsulin processing are noted by increased secretion of proinsulin in type 2 DM. The reasons for the decline in insulin secretory capacity in type 2 DM is still vague. Pancreatic beta cell mass is decreased by 40% in individuals with prolonged type 2 DM. Islet amyloid polypeptide (IAPP) or amylin is secreted by the beta cell along with insulin and this forms the amyloid deposit that are found in the islets of individuals with type 2 DM for longer duration.

In type 2 DM, due to insulin resistance in the hepatocytes, hyperinsulinemia fails to suppress gluconeogenesis, thus resulting in elevated fasting blood glucose and decreased glycogen storage by the liver in the fed state. As a result of insulin resistance in adipose tissue, fat break down and free fatty acid flux from fat cells are increased, leading to increased lipid synthesis in hepatocytes. This storage of fat in the liver lead to nonalcoholic fatty liver disease and abnormal liver function tests, responsible for the dyslipidemia found in type 2 DM [26].

Type 2 DM - A Disease of Fat Metabolism

In type 2 DM, the prime abnormality is an accelerated fat breakdown and decreased synthesis of fatty acids and triglycerides leading on to increased ketone body production. So, it has been considered "more a disease of lipid than of carbohydrate metabolism." In a normal individual after a carbohydrate meal 50% of ingested glucose is burnt to carbon dioxide and water through glycolysis, 30–40% is converted to fat and 5% is stored as glycogen for future use. In type 2 DM, less than 5% of the ingested glucose is converted into fat, in spite of a decrease in the amount burnt to CO_2 and H_2O. But there is no change in the amount converted to glycogen. So, blood glucose level increases and the extra glucose is passed in urine. In diabetes, there occurs an intracellular deficiency of glucose and so conversion of carbohydrates to lipids in the depots is decreased. Insulin inhibits the hormone-sensitive lipase in adipose tissue. So, in the absence of this hormone the plasma level of free fatty acids is doubled. Free fatty acids are mobilized to circulation pool by glucagon and it parallels the plasma glucose level in diabetes.

Free fatty acid levels in blood are a better indicator of the severity of the diabetic state. In the liver and other tissues, the fatty acids are catabolized to acetyl-CoA. Some of the acetyl-CoA is burnt along with amino acids to yield CO_2 and H_2O in the Citric Acid Cycle. However, the supply exceeds the capacity of the tissues to catabolize the acetyl-CoA. Due to (a) excess catabolism of fatty acids, (b) increased gluconeogenesis and (c) marked outpouring of glucose into circulation, conversion of acetyl-CoA to malonyl-CoA and thence to fatty acids is markedly impaired. This happens due to a deficiency of acetyl-CoA carboxylase, the enzyme that catalyzes the above conversion. The excess acetyl-CoA is converted to ketone bodies. In uncontrolled diabetes, the plasma concentration of triglycerides, chylomicrons and free fatty acids are increased, and so the plasma is often lipemic. The rise in these substances is mainly due to decreased activity of lipoprotein lipase which helps in the removal of triglycerides into fat depots.

CALCIUM AND TYPE 2 DIABETES MELLITUS

Ca^{2+} signaling in the β-cell: Calcium is a very essential second messenger in cells, involved in a various cellular processes including cell growth, regulation of genes, cell proliferation, metabolism, exocytosis, and programmed cell death. Calcium levels should be strictly regulated as high levels are toxic to cells. Cytosolic baseline levels of calcium are typically 10,000 times lower than the extracellular environment. The endoplasmic reticulum serves as a major intracellular calcium store. It stores micro molar amounts of the molecule. Calcium is involved in signaling whether initiated extrinsically or intrinsically. These calcium signals can originate from channels on the endoplasmic reticulum, the inositol phosphate-3 receptor, ryanodine receptor, a ligand-gated channel or from the various other channels and pumps on the plasma membrane, or from other organelles such as the golgi apparatus and mitochondria. There are various channels and pumps that buffer calcium, to turn signals off and return the cytoplasmic calcium to normal levels, including sarcoplasmic endoplasmic reticulum calcium ATPase (SERCA) pump. Altogether, multitude mechanisms operate synchronously as a toolkit creating a complex code controlling vast array of cellular processes[27]. Calcium signaling that occur in pancreatic β-cells was studied in detail. The intracellular calcium channels and membrane calcium channels create cytosolic calcium oscillations which create an oscillatory insulin secretion in glucose-stimulated β-cells. The cascade of events leading to glucose-stimulated calcium signaling in β-cells starts when glucose is internalized by the transporter protein. The glucose that has entered the beta cell undergoes anaerobic glycolysis and thus increases ATP/ADP ratio. The increased ATP inhibits K^+ ATPase channel resulting in depolarization and activation of voltage-dependant calcium channels present on plasma membrane. The increase in calcium concentration just beneath the cell membrane regulates docking and fusion of secretory granules, resulting in insulin secretion. This secretion is pulsatile in fashion, and it creates oscillations in plasma insulin levels that are crucial for insulin action on its target tissues[28].

MAGNESIUM AND TYPE 2 DIABETES MELLITUS:

A 70 kg man contains 35 grams of magnesium. About 55-60% of magnesium is combined with calcium and phosphorus in the bones. 20-25% is located in soft tissue and 1% is present in body fluids. The recommended dietary allowance for magnesium is 400-420 mgms in males and 310-320 mgms in females. Magnesium is widely distributed in foods, especially plant foods, because it is part of the chlorophyll molecules. Green vegetables, nuts, legumes, seafood, chocolate, whole grain cereals, coffee, tea and cocoa are rich in magnesium. Magnesium is absorbed throughout the small intestine, mostly in the distal jejunum and ileum and also absorbed in the colon if disease has impaired the small intestine absorption. When a person is taking a normal diet 40-60% of magnesium is absorbed, but a smaller % is absorbed at high intakes and a larger % at low intakes. Serum magnesium concentration in the blood is maintained within a narrow range by the small intestine and kidney. Small intestine and kidney increase their magnesium absorption whenever necessary. If even greater amounts are required, the bones can release some magnesium into the extra cellular compartment. Excess magnesium is eliminated by the kidneys. Such excretion can be increased by protein, alcohol, and caffeine consumption[29].

Calcium and phosphorus inhibit magnesium absorption. High zinc intake may be a health concern for individuals consuming less magnesium. In addition, calcium and magnesium compete with each other for reabsorption in kidneys. Inadequate dietary intake usually does not cause deficiency rather it is the increased excretion results in magnesium deficiency[30].

Magnesium participates in greater than 300 essential metabolic reactions. Magnesium is necessary for the transmission of nerve impulses and the relaxation of skeletal muscles after contraction. Magnesium activates the enzymes that add the 3rd phosphate group to ADP to form ATP. It activates enzymes for the metabolism of carbohydrates, fats, and proteins, including protein synthesis. It also helps in the release of energy from muscle glycogen. Magnesium acts a cofactor in calcium utilization for bone formation and also helps to hold calcium in tooth enamel, thus preventing tooth decay[31].

Magnesium deficiency is a common occurrence, especially in older people with decreased oral intake, people with chronic alcoholism and those who use diuretics. In a study of emergency department clients on whose serum magnesium was determined, 31% had low magnesium and this was statistically associated only with pregnancy and diabetes mellitus. Insufficient magnesium impairs central nervous system activity and increases muscular excitability because magnesium metabolism is intricately linked to calcium metabolism. Magnesium deficient clients display the signs of tetany. Other signs include disorientation, convulsions, psychosis. Relief of signs and symptoms may take 60-80 hrs after treatment begins[32].

Magnesium deficiency has been associated with insulin resistance, carbohydrate intolerance, cardiac arrhythmias, congestive heart failure, retinopathy and hypertension. The most common cause of hypomagnesaemia in diabetes mellitus is loss of magnesium through chronic glycosuria and diuretic use. A deficiency may lead to insulin resistance or may be a result of insulin resistance. Several studies have found that supplementation with magnesium can improve glucose control and insulin sensitivity. The American Diabetes association does not recommend routine evaluation of magnesium status in healthy individuals with diabetes but recommends routine evaluations for those people at high risk for magnesium losses, such as those with poor glycemic control, those receiving diuretics, those with intestinal malabsorption, those with calcium or potassium deficiencies and pregnant women[33].

GLYCOSYLATED HEMOGLOBIN (HbA1c):

Glucose is bound to hemoglobin irreversibly by a continuous slow non enzymatic process resulting in the formation of glycosylated hemoglobin.

The percentage amount of hemoglobin that undergoes glycosylation to form HbA1c is directly related to the average concentration of glucose in the blood. In a normal person about 3-6 gm% of hemoglobin is glycosylated. In a diabetic the glycosylated hemoglobin may double or even triple. HbA1 is comprised of atleast 3 distinct sub-fractions: HbA1a, HbA1b and HbA1c. HbA1c is the major component and the fraction of clinical importance. The significance of HbA1a and HbA1b sub-fractions are not known and their correlation with long term glucose control is very questionable. Glucose combines by ketamine linkage to N-terminal valine as well as with lysine residues of both alpha and beta chains of hemoglobin. This reversible adducts (pre-HbA1c) can then undergo an amadori rearrangement to stable ketoamine (HbA1c). Pre-HbA1c formation will follow rapidly and proportionately to the amount of available glucose[34]. The circulating erythrocytes have a half-life of 60 days. So, HbA1c values will begin to reflect radical changes in diet or other modes of therapy approximately 3-4 weeks after the initiation of diet or therapy. Unlike urine and blood glucose tests, HbA1c measurement is not a momentary look at sugar levels but an indicator of the average blood sugar concentration over the previous two or three months. HbA1c has the advantage of not influenced by diet, mode of therapy, physical activity, relations to meals and patient co-operation at the time of testing. HbA1c values may be misleading in conditions where life span of red blood cells is altered as in hemolytic disorders and hemoglobinopathies. Glycosylated hemoglobin is not useful for day to day management and in adjusting the dose of insulin or oral anti-diabetic drugs. Along with HbA1c, blood glucose values must be available to the physician. It can be useful to distinguish stress hyperglycemia from pre-existing diabetes in situations of hyperglycemia associated with conditions like infection, myocardial infarction, stroke and surgery[35].

EFFECT OF TYPE 2 DM ON PERIPHERAL NERVES:

Diabetic neuropathies have multifactorial pathogenic mechanisms, and varied clinical presentation. Chronic hyperglycemia plays a dominant role in the pathogenesis of diabetic neuropathy. Other metabolic consequences of hyperglycemia such as increased polyol pathway activity, myo-inositol depletion and Na^+K^+ATPase activity contribute to the pathogenesis of diabetic neuropathies. These pathogenic factors operating either individually or in combination contribute to the syndrome of diabetic neuropathy.

The accumulation of sorbitol has deleterious effects on nerve conduction velocity. This is attributed to Schwann cell damage caused by increase in osmolarity due to sorbitol and fructose. Hyperglycemia causes increased intracellular concentrations of glucose, resulting in increased activity of polyol pathway. This acts through unknown mechanism leading to depletion of myo-inositol concentration which inhibits tissue Na^+ K^+

ATPase activity. The synthesis of myelin which is rich in cholesterol, cerebroside and sphingomyelin and phosphotidyl serine-phosphotidyl inositol is likely to be reduced contributing to neuropathy.

The normal vascular autoregulation is lost leading on to decreased endoneural blood flow and hypoxia. Though vascular and metabolic hypothesis for the pathogenesis of diabetic neuropathy has been postulated individually, more often there is an overlap of both metabolic and vascular events. Endothelial proliferation with luminal narrowing, hyperplasia of pericyte basement membrane and thickening of the perivascular space are frequently observed. Metabolic and vascular factors, together with glycosylation of proteins, hyperaggregation of platelets may all contribute to the development of neuropathy.

ELECTROPHYSIOLOGICAL ABNORMALITIES:

Nerve conduction studies are of great assistance in detecting neuropathy without having to resort to histopathological studies. In addition, conduction studies are extremely useful in confirming presence of focal nerve entrapments (carpal tunnel, cubital tunnel and common peroneal tunnel syndromes etc.). Newer techniques give a fairly accurate assessment of extent of neuropathy. Needle electromyography is a more recent and sophisticated test. Sensory nerve action potential and autonomic function tests are more sensitive in identifying subtle abnormalities of nerves in diabetic neuropathy than motor conduction studies. Sequential EMG changes after axonal damage ranging from reduced recruitment of motor unit potentials occurring as early as 7 days to the presence of fibrillation potential, polyphasic high amplitude longer duration motor unit potentials, absent F waves reflecting changes occurring later in the course of the disease are recorded[35].

COMPLICATIONS OF TYPE 2 DM

Complications of type 2 DM is broadly classified into acute and chronic.

Acute complications

Diabetic ketoacidosis and Hyperglycemic Hyperosmolar state

Chronic complications are classified into

Microvascular and Macrovascular

Microvascular complications

Eye disease -Retinopathy, Macular edema

Neuropathy -Sensory and Motor neuropathy, Autonomic neuropathy

Nephropathy

Macrovascular complications

Coronary artery disease, Peripheral arterial disease and

Cerebrovascular disease

Others- periodontal disease, cataract, glaucoma, sexual dysfunction gastroparesis, skin and systemic infections.

The risk of chronic complications increases as the duration and degree of hyperglycemia increases. Randomized clinical trials in type 2 DM have demonstrated that a reduction in chronic hyperglycemia prevents or delays retinopathy, neuropathy, and nephropathy. Some individuals with long-standing DM never develop nephropathy or retinopathy. This precludes that there is some genetic susceptibility for developing particular complications. Coronary heart disease and related mortality rates are two to four times greater in patients with type 2 DM. These events correlate with fasting plasma glucose, postprandial plasma glucose, and HbA1c, hypertension and lipid levels[36].

THEORIES EXPLAINING THE EFFECT OF CHRONIC HYPERGLYCEMIA:

(i) The increased intracellular glucose leads to the formation of advanced glycosylation end products, which bind to a cell surface receptor through non-enzymatic glycosylation. Non-enzymatic glycosylation results from the interaction of glucose with amino groups on proteins. These products cross-link proteins such as collagen, extracellular matrix proteins and accelerate atherosclerosis, promote glomerular dysfunction, reduce nitric oxide synthesis, induce endothelial dysfunction, and alter extracellular matrix composition and structure.

(ii) A second theory is that chronic hyperglycemia increases glucose metabolism via the sorbitol pathway. Intracellular glucose when it reaches a level above which glycolysis is impossible within the cells, is converted to sorbitol by the enzyme aldose reductase. Sorbitol accumulates inside the cell and alters redox potential, increases cellular osmolality, generates reactive oxygen species, and ultimately leads to cellular dysfunction. Aldose reductase inhibitors have no proven effect on the complications of diabetes mellitus.

(iii) A third theory is that chronic hyperglycemia increases the formation of diacylglycerol. Diacylglycerol activates protein kinase C which causes transcription of genes for fibronectin, type IV collagen, contractile proteins, and extracellular matrix proteins in endothelial cells and neurons.

(iv) Chronic hyperglycemia generates fructose-6-phosphate through hexosamine pathway. This alters function of cell by glycosylation of endothelial nitric oxide synthase and by changes in gene expression of transforming growth factor and plasminogen activator inhibitor-1.

Vascular endothelial growth factor-A, platelet-derived growth factor, epidermal growth factor, insulin-like growth factor I, growth hormone, basic fibroblast growth factor, and even insulin, have been suggested to play a role in DM-related complications.

NEUROPATHY IN DIABETES MELLITUS

STAGES OF DIABETIC PERIPHERAL SENSORY NEUROPATHY:

Stages of neuropathy	**Characterisitics**
No neuropathy	No symptoms or signs
Clinical neuropathy Chronic painful	 Burning/shooting/stabbing pain;±pins and needles; increased at night; absent sensation to several modalities; reduced/ absent reflexes
Acute painful	Severe symptoms as above; may follow initiation of insulin in poorly controlled diabetes; signs minor or absent
Painless with complete/partial sensory loss	Numbness/deadness of feet or no symptoms; painless injury; reduced/absent sensation; reduced thermal sensitivity; absent reflexes
Late complications	Foot lesions; neuropathic deformities; non-traumatic amputations.

CLINICAL FEATURES OF DISTAL SENSORIMOTOR DIABETIC NEUROPATHY

Large fibre type	**Small fibre type**
Unsteady gait	Pain predominates
Absent reflexes	Variable reflexes
Decreased vibration/position sense	Variable position/vibration sense
Charcot’s joints possible	Variable presence of Charcot’s joints
Mimics posterior column lesions	Ultimately leads to sensory loss

AUTONOMIC NEUROPATHY

Conventionally, diagnosis of autonomic neuropathy is based on simple tests using cardiovascular reflexes during deep breathing, standing, Valsalva maneuver and blood pressure response to standing and sustained handgrip. Tests involving parasympathetic nerves show abnormalities earlier than those involving sympathetic. Loss of sweating in the feet and impotence may precede abnormal cardiovascular tests. Diabetic with autonomic neuropathy are prone for sudden death from painless myocardial infarction and cardiorespiratory arrest. Generally, symptoms due to autonomic neuropathy include postural hypotension, gustatory sweating, diarrrhoea, unawareness of hypoglycemia; bladder dysfunction, impaired temperature regulation and altered sweating. Delayed gastric emptying, marked retention and patulous pylorus(gastroparesis diabeticorum), disorder of esophageal motility, bladder paralysis resulting in urinary retention with superimposed infection and sexual dysfunction contribute significantly to the morbidity in subjects with autonomic neuropathy. Pupillary abnormalities namely a reduction in the resting pupil size, loss of spontaneous oscillations (hippus), loss of light reflex and occasionally loss of accommodation reflex are observed. Very rarely, a true Argyll-Robertson pupil is seen[35].

RELATED STUDIES

Chandrashekar M Sultanpur et al[37] did a review on glycosylated hemoglobin in the diagnosis of diabetes mellitus. A normal non-diabetic HbA1c is 3.5-5.5%.The IDF and American College of Endocrinology recommend HbA1c values below 6.5%, while ADA recommends that the HbA1c to be less than 7.0% for most patients. On average, HbA1c of 6% corresponds to mean plasma glucose of 135 mg/dl of blood. Vitamins C and E have been reported to lower HbA1c measurements by inhibiting glycosylation. Alcoholism, lipidemia, and chronic ingestion of salicylates, is also known to reduce the level of HbA1c. Higher levels of HbA1c level can be seen in people with a longer red blood cell life span, such as with Vitamin B12 or folic acid deficiency. There is also some evidence of wide fluctuations in HbA1c between individuals. There are two groups based on this called as “low glycators” and “high glycators”. When the HbA1c and fructosamine levels are measured simultaneously, HbA1c does not accurately reflect glucose control. So measurement of fructosamine was suggested to be routinely used in clinical practice. They also added that HbA1c level cannot be used as a sole tool for assessing the diabetic status. The additional peaks that occur in HPLC’s lead to overestimation and underestimation of HbA1c results. Electrospray mass spectrometry and a method based on quenching of the fluorescence of an eosin-boronic acid solution ate newer methods. The immunoagglutination method called the DCA 2000 uses a specific antibody against the first six amino acid residues of the glycated N-terminal of hemoglobin. This method suggests that only few hemoglobin variants are known to interfere with HbA1c results.

Firas Salih Abdul Hadi et al[38] did a study to investigate the effects of type 2 DM on the levels of HbA1c, testosterone and calcium with relationship of leptin on 45 diabetic male patients and 20 apparently healthy controls. It was conducted at Specialist Center for Endocrine and Diabetes Diseases in Baghdad province. The age of men ranged 25-54 years. Calcium levels showed significant ($p<0.05$) decreasing (2.26 ± 0.03) in non-obese T2DM in comparison with control group (2.39 ± 0.03) and obese T2DM (2.30 ± 0.04) but the value was within normal range (2.0-2.6mmol/l).

Rajni Dawar Mahajan et al[39] reviewed several published articles to analyze the importance of using HbA1c for diagnosing Diabetes mellitus in India. At the end of their review they concluded that in the present Indian scenario Glycated hemoglobin should not be accepted as an independent test to diagnose type 2 DM. A similar analysis was done by **Chris Florkowski**[40]. The study concluded that HbA1c can be a diagnostic test provided it should be measured in a laboratory using a National Glycohemoglobin Standardization Program-certified assay, and also stressed that in the absence of unequivocal hyperglycemia the test should be repeated.

Zaccardi F et al[41] did a prospective study in which ionized calcium and cardio metabolic risk factors were identified in a population-based sample of 2350 men without a known history of type 2 DM at the start. The associations between ionized calcium levels and incident cases of type 2 DM were estimated using Cox regression analyses adjusted for potential confounders. After a median follow-up of 23.1 years, 140 new cases of type 2 DM were recorded. A multivariable analysis adjusted for age, BMI, systolic blood pressure, serum HDL-cholesterol, and family history of type 2 DM was done and they could not find an association between ionized calcium and future risk of type 2 DM.

Kanchana et al[42] in their comparative study of calcium and vitamin D levels in diabetics and non-diabetics have found an inverse relationship between serum calcium and blood glucose level.

D.S. Pushparani et al[43] in their research evaluated the serum level of calcium and iron in type 2 diabetes mellitus with periodontitis subjects. A total of 450 subjects participated in the study. They were equally distributed into three groups as control healthy individuals, type 2 DM without periodontitis, and type 2 DM with periodontitis. They were matched for age, sex, and duration of diabetes mellitus. According to Newman-Keuls Multiple Comparison test, the means levels of serum Calcium of group II was lesser than the means of all other groups. The mean calcium level in type 2 DM with periodontitis (group III) was significantly higher when compared to control. However, in the case of type 2 DM without periodontitis, the serum Ca levels were decreased non-significantly.

Anubha Shukla et al[44] reviewed the significance of Calcium and Vitamin C in maintaining glucose homeostasis. They pinned that the increased blood glucose levels can inhibit calcium entry into cells thus causing decreased insulin secretion.

Mohamed Murtuza Kauser et al[45] in their study compared 50 diabetics and 50 non-diabetics to identify serum magnesium levels. The serum magnesium levels in the Oral Hypoglycemic Agent group, insulin group and the insulin plus Oral Hypoglycemic Agent group were 2.02 mg/dl, 1.59 mg/dl and 1.25 mg/dl respectively. The study showed significant decrease in serum magnesium in type 2 DM as compared to controls. The conclusion was hypomagnesaemia may be a contributing factor for the long term complications particularly relating to eyes and heart.

Prabhu G et al[46] had done a prospective study in 132 Type 2 DM patients. Serum magnesium level was estimated and compared with the age matched control group. In their study the serum magnesium levels varied between 1.1 to 3.1 mg/dl against the normal laboratory value of 1.5 -2.5 mg/dl. 33(25%) of the study group had low serum magnesium levels(less than or equal to 1.5mg/dl). This led to an assumption that serum magnesium was lower in patients when the duration of the disorder increased. The correlation between hypomagnesaemia and poor glycemic control was statistically significant.

Pramod P Rao and Mohamed Ghouse Shariff's **study**[47] was to know the status of serum magnesium in microalbuminuric and normoalbuminuric Type 2 DM patients. 100 patients of Type 2 Diabetes mellitus attending outpatient/inpatient clinic were grouped into equal groups of 50 patients into microalbuminuria and normoalbuminuria. Fasting blood sugar, postprandial blood sugar, HbA1C, renal function test, spot urine albumin creatinine ratio, serum electrolytes including Magnesium levels were compared in both groups. About 6% of microalbuminuria had hypomagnesemia, and one patient had hypomagnesemia. They concluded that low magnesium levels were significantly associated with poor glycemic control and microalbuminuria levels were higher when compared to patients with normal magnesium levels. Retinopathy was also significantly associated with hypomagnesemia. Therefore, screening for serum magnesium levels in Type 2 DM and its correction may help in achieving better glycemic control, which can prevent further diabetic complications.

In a study by **Sasmita Mishra et al**[48] there was a positive correlation between high density lipoprotein and serum magnesium. There happened to be a negative correlation between low density lipoprotein, triglycerides and serum magnesium in the type 2 DM subjects.

Serum magnesium levels were compared between pre-diabetics and healthy age matched individuals in the study by **K. Sumathi and A. Mary Chandrika**[49]. They identified hypomagnesemia in diabetics and proposed the value of magnesium supplementation in type 2 DM. **S. Ramdass et al**[50] categorized the study population of type 2 DM subjects into three types based on their glycemic control and estimated serum magnesium levels. It was found that magnesium was reduced in the group which had a poor control in the blood glucose levels.

Abhijeet A. Adgaonkar et al[51] in their study “Clinical Profile of Peripheral Neuropathy in Diabetes Mellitus by Nerve Conduction Study” included 50 subjects with type 2 DM. They included subject from both sex and different age group ranging from 20 to 70 years with varying period of disorder. They assessed involvement of peripheral nerves both clinically as well as with nerve conduction study. They found that the incidence of peripheral neuropathy was recorded as 30% on clinical examinations. Whereas on nerve conduction study it was found 42 %. They concluded that diabetic peripheral sensory motor peripheral neuropathy was the commonest type of presentation and the lower limbs were almost always affected and the upper limbs were less affected. The similar study by **Yacoub G Bahou et al**[52] also had the similar presentation and results.

Arindam Dutta et al[53] did a study entitled “Prevalence of peripheral neuropathy in newly diagnosed type 2 diabetics”. 100 newly diagnosed type 2 diabetic patients attending the Diabetes clinic were taken for the study. Multiple logistic regression analysis showed that duration of diabetes had highest contribution and age, and blood glucose have some contribution. The prevalence of peripheral neuropathy in newly diagnosed type 2 diabetic patients using clinical and electrophysiological methods was 29%. They ended that there was a significant correlation between peripheral neuropathy and duration of diabetes, age of the patients and fed-state blood glucose levels.

Kanavi Roopa Shekharappa et al[54] did a study on “The Utility of Nerve Conduction studies in type 2 DM”. 45 patients with normal HbA1c and 45 patients with elevated HbA1c levels were included. Analysis revealed that conduction of nerves progressively decreased from patients with good glycemic control to those with poor glycemic control. They concluded that the nerve conduction studies can be employed for diabetic patients who have early neuropathic symptoms.

Vijay Viswanathan et al[55] did “Nerve conduction abnormality study in different stages of glucose intolerance” in a Diabetes Research Centre. 225 subjects were categorized into normal glucose tolerance, impaired glucose tolerance and asymptomatic newly diagnosed type 2 DM patients. Motor and sensory nerve conduction velocities were measured. The motor nerve conduction velocity demonstrated by IGT subjects were slower and so they concluded that type 2 DM subjects need an early screening for complications.

MATERIALS AND METHODS

STUDY DESIGN: This was a cross-sectional study.

STUDY CENTRE AND PERIOD: This was conducted in Tirunelveli medical college Hospital from November 2015 to August 2016.

SAMPLE SIZE: The sample size was 75.

STUDY GROUP: The study group was divided into two categories.

- First group - Newly diagnosed type 2 DM patients which included 25 female and 20 male patients
- Second group – Type 2 DM patients who were taking treatment for more than five years which included 15 female and 15 male patients.

ETHICAL CONSIDERATIONS:

Approval from institutional ethical committee of Tirunelveli Medical College was obtained. The procedure was explained in detail to all subjects in their own local language. Written informed consent was obtained from those persons who were willing to undergo this study.

INCLUSION CRITERIA:

- Newly diagnosed type 2 DM patients- involved male and female patients who were diagnosed to have type 2 DM for less than a year and were on regular treatment.
- The other group was male and female patients with type 2 DM who were diagnosed to have the disease for more than 5 years and was on regular treatment.

EXCLUSION CRITERIA:

- Type 2 DM patients who were on irregular treatment.
- Hypertensive patient
- Patients with preexisting neurological disorders
- Pregnant women
- Smoking and alcohol intake
- Use of drugs causing neuropathy
- Liver and kidney disease
- Family history of neuropathy

Each person had a separated format wherein personal details, family history and history of illnesses were recorded. It also included anthropometric measurements.

ERBA CHEM – 5 SEMI-AUTOMATED ANALYZER

COLLECTION OF VENOUS BLOOD SAMPLE

STUDY METHODS:

First, the patients were interrogated about their personal history, medical history, treatment history and family history relating to type 2 DM. Second, they were taken to the Central Diagnostics Laboratory for blood collection. Patients were asked to relax and be seated comfortably. Then, following universal precautions 5 ml of blood sample was obtained from antecubital vein for estimation of serum magnesium, serum calcium, HbA1c. Serum was separated using a centrifugation machine for estimation of calcium and magnesium.

Subsequently they were directed to the Neurophysiology Lab for nerve conduction study.

- HbA1c (ERBA) – Particle enhanced Immuno-turbidimetric method was employed. IFCC reference method is used. It is applicable for hemoglobin values ranging from 6-26 gm%. HbA1c measuring range was 3.98-15.42%. HbA1c was determined using CHEM-5 semi-automated analyzer.
- Calcium (LABCARE)- Calcium with Arsenazo III at neutral pH yield a blue coloured complex. The intensity of colour was proportional to calcium levels. Serum reference values 8.8-10.2 mg/dl. Serum calcium was determined using CHEM-5 semi-automated analyzer.

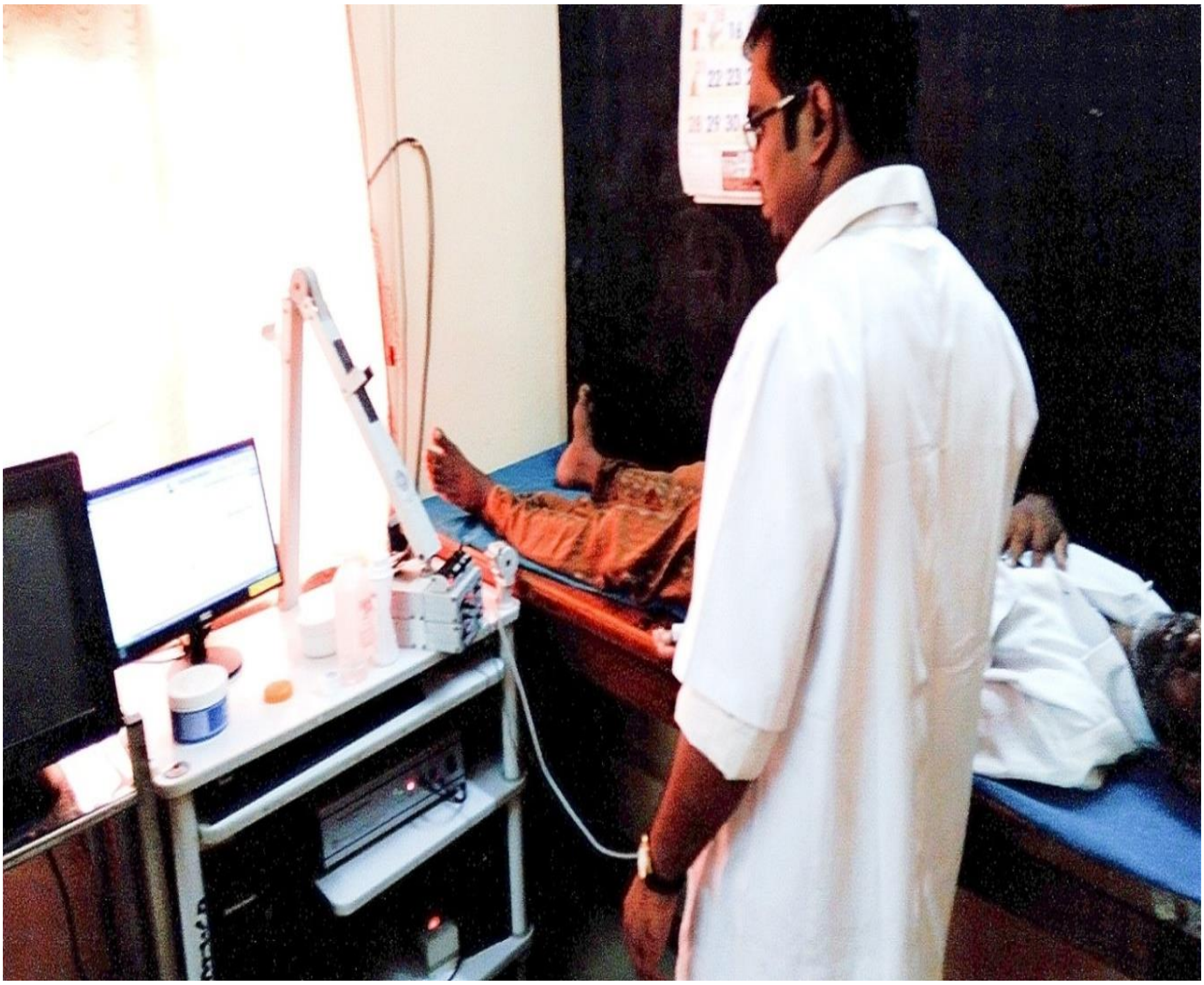
- Serum magnesium was determined photocolorimetrically using kit method in CHEM-5 semi-automated analyzer. The normal range is 1.9-2.5 mg/dl.

BASIC COMPONENTS OF NERVE CONDUCTION RECORDING EQUIPMENTS:

Electro diagnostic equipments consist of both stimulating and recording systems, amplifiers, filters, microprocessor and video and audio monitors in addition to computers.

STIMULATING SYSTEM:

The primary function of a nerve is the transmission of an electrical impulse from one source point to another. The stimulus usually travels from the nerve cell body or from sensory receptors. But, in nerve conduction studies the nerve is stimulated by an external electrical source. When the nerve is near the surface of the body, skin electrodes are enough to record the electrical impulse. Whereas, deeper nerves require needle electrodes. During surgery, stimulating electrodes are applied directly over the exposed nerves. To obtain a maximal response, all nerve fibres are stimulated by using supra maximal stimulus. Sub maximal stimulus gives rise to false results.



NERVE CONDUCTION STUDY

RECORDING SYSTEM:

The electrodes are

- Surface electrodes
- Needle electrodes.

There are three types of surface electrodes. They are disc, cup and ring electrodes. Disc electrodes are used in nerve conduction studies and motor evoked potentials and cup electrodes in other evoked potentials. Needle electrodes are employed in electromyography and in some situation in nerve conduction and evoked potential studies. The electrodes are made with various types of metal and alloys. Silver chloride electrodes record noise free stable polarization potentials. Three electrodes are used as the components of the recording systems in neuro physiological studies. They are active, reference and ground electrodes. Reference electrode is placed over the tendons. Between stimulator and the recording electrodes the ground electrode is applied. Both active and reference electrodes are kept over the nerves in sensory nerve conduction studies. Amplitude of CMAP or SNAP is altered if the distance between the active electrode and reference electrode is changed. The recommended inter electrode distance in sensory conduction studies is 4 cms.

PARAMETERS MEASURED IN NERVE CONDUCTION STUDY:

In both motor and sensory conduction studies,

• Latency

• Amplitude

• Conduction velocity are the parameters used for interpretation of results.

NERVE CONDUCTION STUDY:

The study subjects were explained about the procedures. The nerve conduction study was performed in left median nerve of upper limb and left sural nerve of lower limb to assess sensory components. The nerve examined for the motor nerve conduction study was left median nerve.

EQUIPMENT USED:

The nerve conduction study was done using **RMS**(Recorders and Medicare Systems) **EMG EP MARK II** at Neurophysiology unit of the neurology department. Surface electrodes were used. For motor nerve conduction study, the low filter-2Hz and high filter-3 kHz was used. For sensory nerve conduction study the low filter-20 Hz and high filter-3 kHz was used. The room temperature was maintained between 23-25 degree celcius. Initially nerves were stimulated with low voltage strength of current and gradually increased till we obtained a maximal response curve.

PREPARATION:

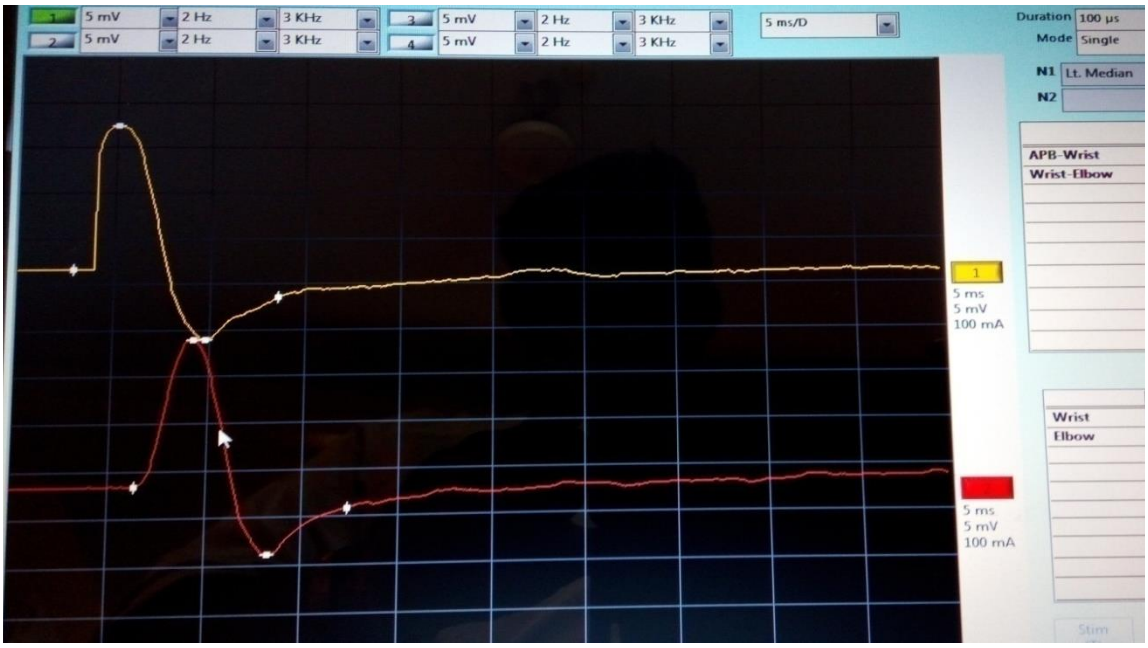
The skin was cleaned with the spirit and allowed to dry. Skin preparation is essential to provide a good contact between the skin and the electrodes and to eliminate artifacts. The electrodes were soaked in normal saline to minimize skin resistance thereby facilitating conduction. Adequate electrode gel should be applied to the recording electrodes used in motor conduction study while fixing it to the skin. Correct placement of electrodes is also important.

SENSORY CONDUCTION STUDY:

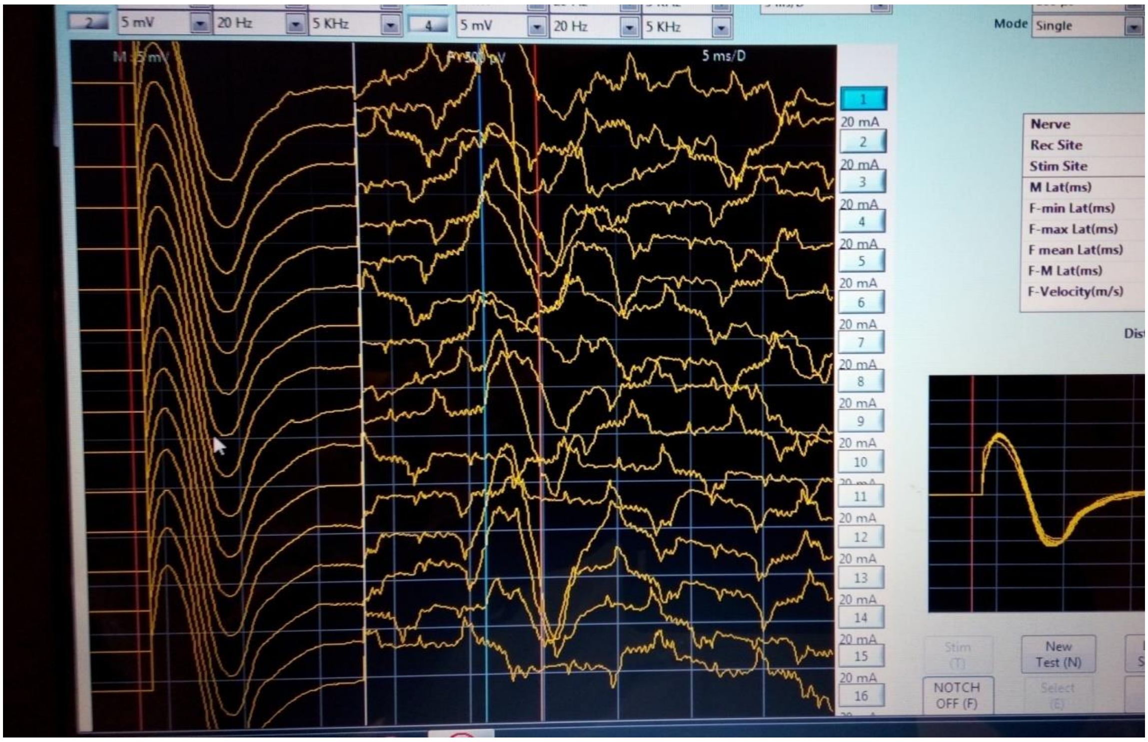
The electrodes were attached to their respective site after preparation of both skin and electrodes and the nerves were stimulated at a single site .The recording electrodes were ring electrodes. The antidromic sensory conduction study was done.

MEDIAN NERVE (Sensory):

1) The site of placement of active electrode was proximal inter phalangeal joint of the left second finger.
2) The reference electrode was placed on the last phalanx of the same finger.
3) The ground electrode was over the dorsum of the hand.



MEDIAN NERVE (MOTOR) CONDUCTION GRAPH



F-WAVE

4) Nerve stimulation was carried out at the wrist between the tendons of palmaris longus and flexor carpi radialis , at the 2^{nd} distal most creases.
5) Peak latency of less than 3.1 ms and minimum conduction velocity and amplitude of 50 m/sec and 30 μv respectively were taken as normal parameters.

SURAL NERVE (Sensory):

1) Active electrode was placed between the left lateral malleolus and the achilles tendon at the malleolar level
2) The reference electrode was placed 3 cm distal to the active electrode.
3) Nerve stimulation was done at a point which is distal to the lower border of the bellies of the gastrocnemius muscle but 10 to 16 cm above the lateral malleolus just lateral to the midline.
4) We used maximum latency of 4.0 ms and minimum conduction velocity of 48 m/sec and minimum amplitude of 8 μv as normal values.

MOTOR CONDUCTION STUDY:

After adequate preparation, the recording electrodes were attached to the correct position with reference to the nerve to be studied. The nerves were stimulated in two points. The distance between the two points of stimulus application was measured and entered for calculation of conduction velocity.

MEDIAN NERVE:

1. Active electrode was placed over Abductor pollicis brevis

2. Reference electrodes were placed over the muscle tendon

3. Ground electrode was kept over the dorsum of hand.

4. Nerve was distally stimulated at the wrist between the tendons of palmaris longus and flexor carpi radialis tendons at the second crease.
5. For proximal stimulation of nerve, the stimulator was placed at the elbow crease, medial to the biceps tendon and brachial artery.
6. Normal values are maximum onset latency of 3.4 ms and minimum conduction velocity and amplitude of 53m/s and 4 mV respectively.

F wave latency was recorded by stimulating the nerve at the distal point to get 10 to 20 F waves[56].

STATISTICAL ANALYSIS

Databases of all personal details such as age, sex, weight and height, laboratory values such as HbA1c, serum calcium, serum magnesium and nerve conduction study values such as latency, conduction velocity and amplitude for the peripheral nerves under study were entered in Microsoft Excel sheet and a master chart was prepared. Statistical analysis of all study databases were performed by IBM SPSS version 20.0 software.

Mean (SD) and frequency (percentage) were used to describe the summary data. Two sample ‘t’ test was used to compare the mean difference between groups. Pearson correlation analysis was used to check the correlation between nerve conduction parameters such as latency, conduction velocity and amplitude with laboratory parameters. ‘P’ value less than 0.05 was considered as statistically significant.

Table 1: Age distribution among the two study groups

Age(years)	Mean(SD)	Range	p value
Group I (n=45)	45.60(4.2)	38 – 56	<0.001*
Group II (n=30)	51.27(7.0)	38 – 65	

***p value< 0.05 is statistically significant

Figure: Mean of age distribution in the two study groups

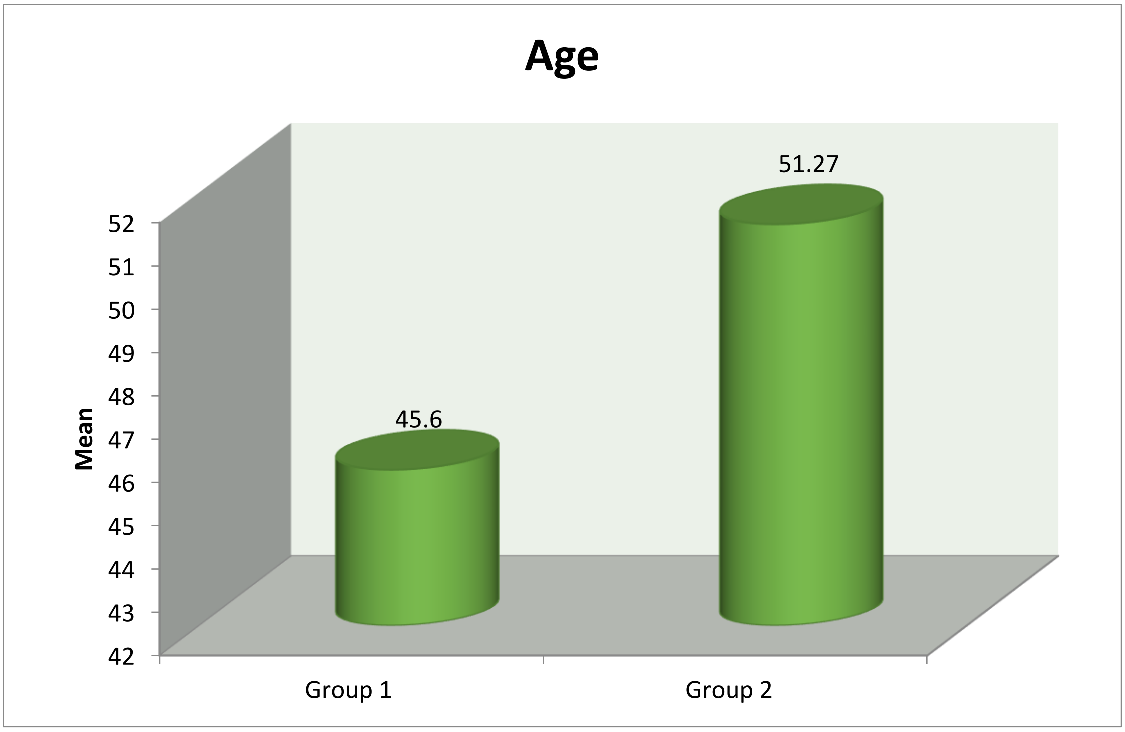


Figure: Percentage distribution of sexes in the two groups

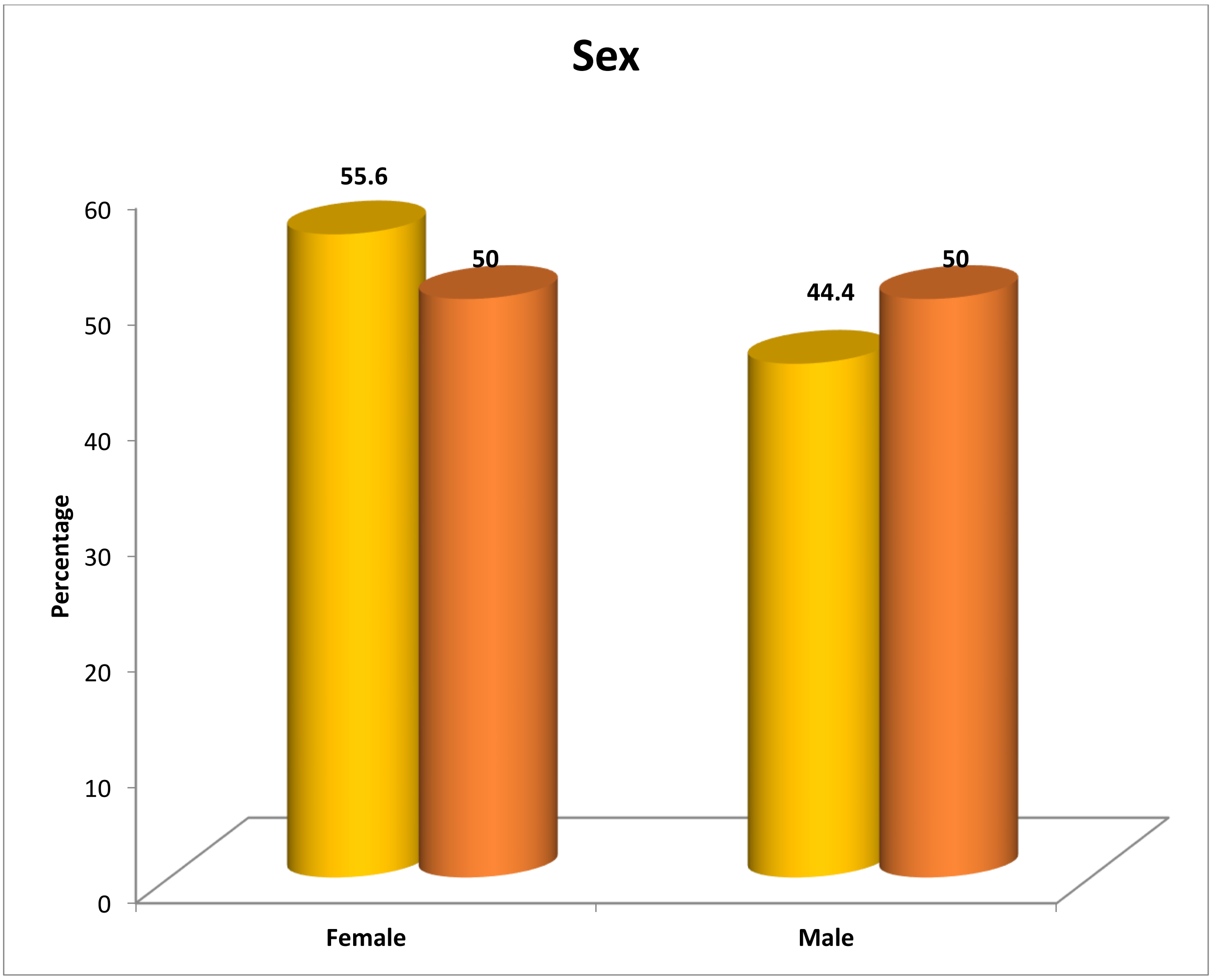


Table 2: Comparison of means of HbA1c, Serum Magnesium and Serum Calcium

Variable	Group1 (n=45)		Group2 (n=30)		p value
	Mean (SD)	Range	Mean (SD)	Range	
HbA1c	6.37(0.8)	4.8 – 7.9	7.09(0.9)	4.6 – 8.9	<0.001*
$Sr.Cal^{2+}$	9.05(0.7)	8.0 – 10.5	9.07(0.6)	8.0 – 10.6	0.897
$Sr.Mg^{2+}$	2.21(0.3)	1.5 – 2.9	1.94(0.3)	1.4 – 2.5	0.001*

*p <0.05 is statistically significant

Figure: Mean of HbA1c in the two groups compared with bar diagram

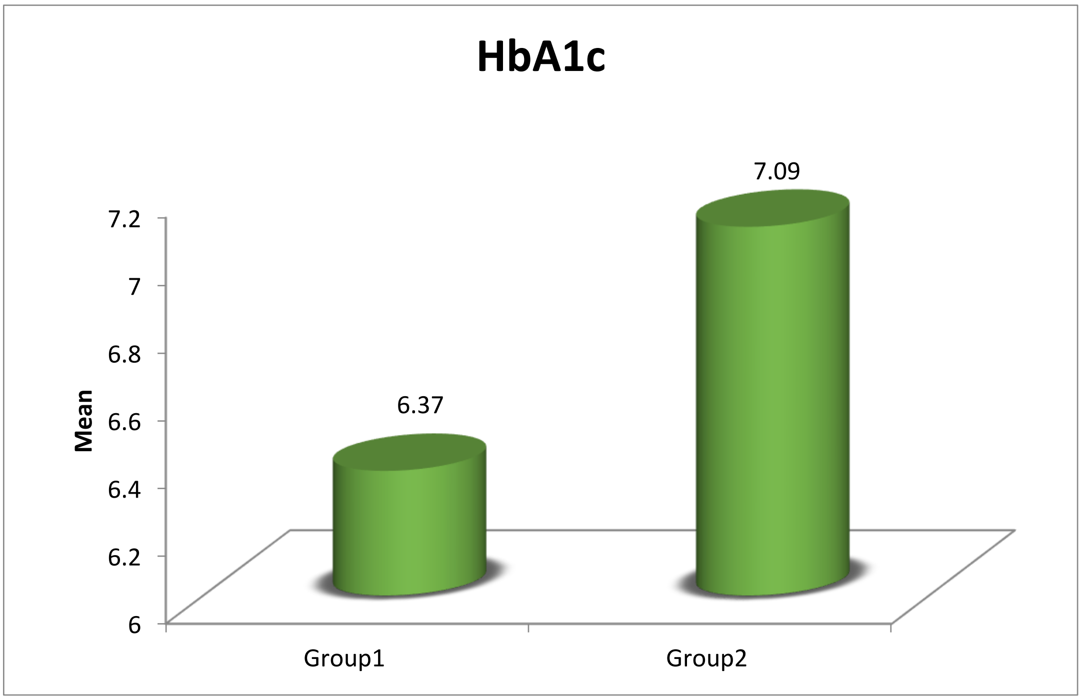


Figure: Comparison of means of serum calcium in the two groups

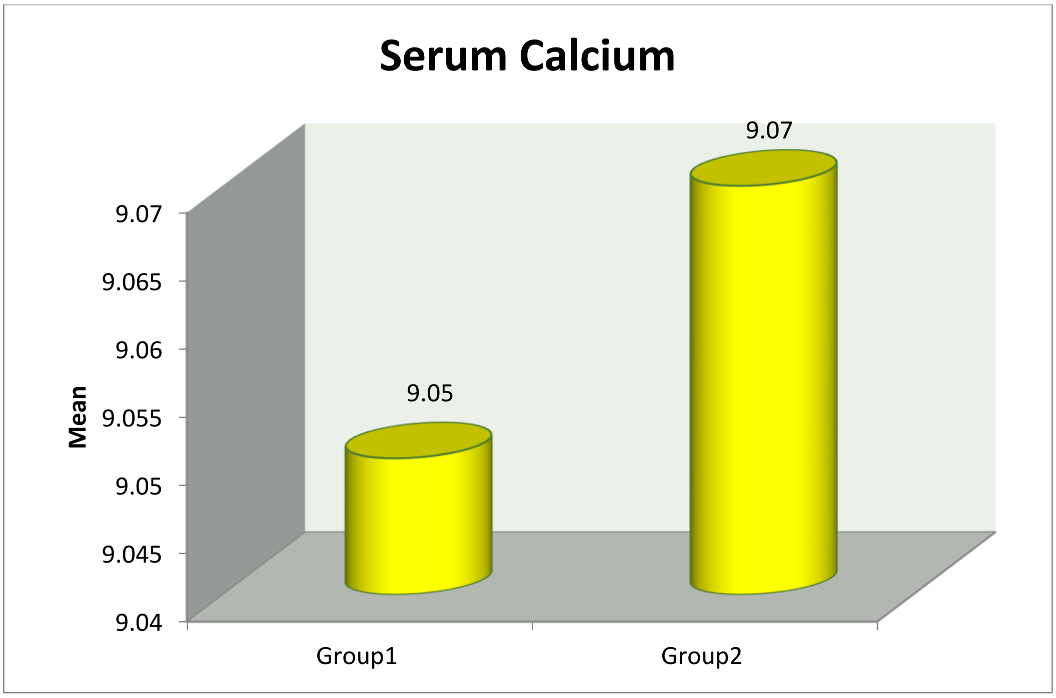


Figure: Comparison of means of serum magnesium in the two groups

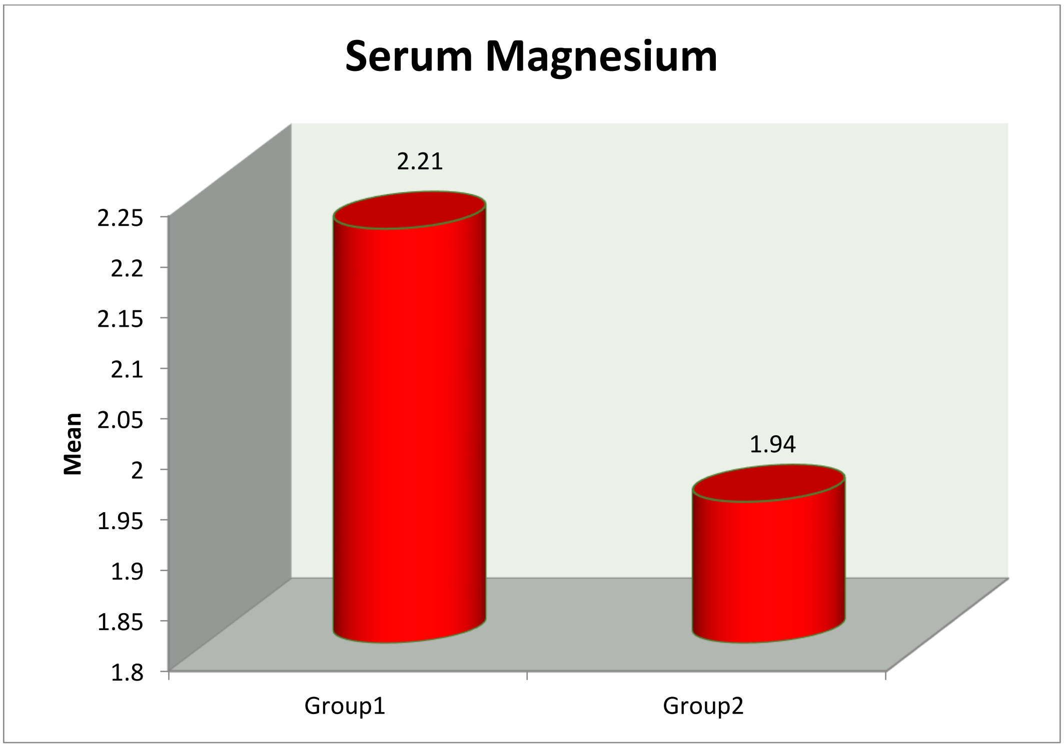


Table 3: Parameters of Median Nerve (Sensory) conduction

Median Nerve Sensory	Group1 (n=45)		Group2 (n=30)		p value
	Mean (SD)	Range	Mean (SD)	Range	
LAT	2.31(0.2)	1.95 – 2.84	2.61(0.5)	2.03 – 3.65	0.001*
AMP	34.74(2.8)	30.25 – 40.45	32.18(2.0)	28.54 – 35.86	<0.001*
CV	56.16(1.7)	52.12 – 59.43	52.36(1.9)	48.75 – 55.67	<0.001*

*p <0.05 is statistically significant

Figure: Median nerve (Sensory) conduction parameters between groups

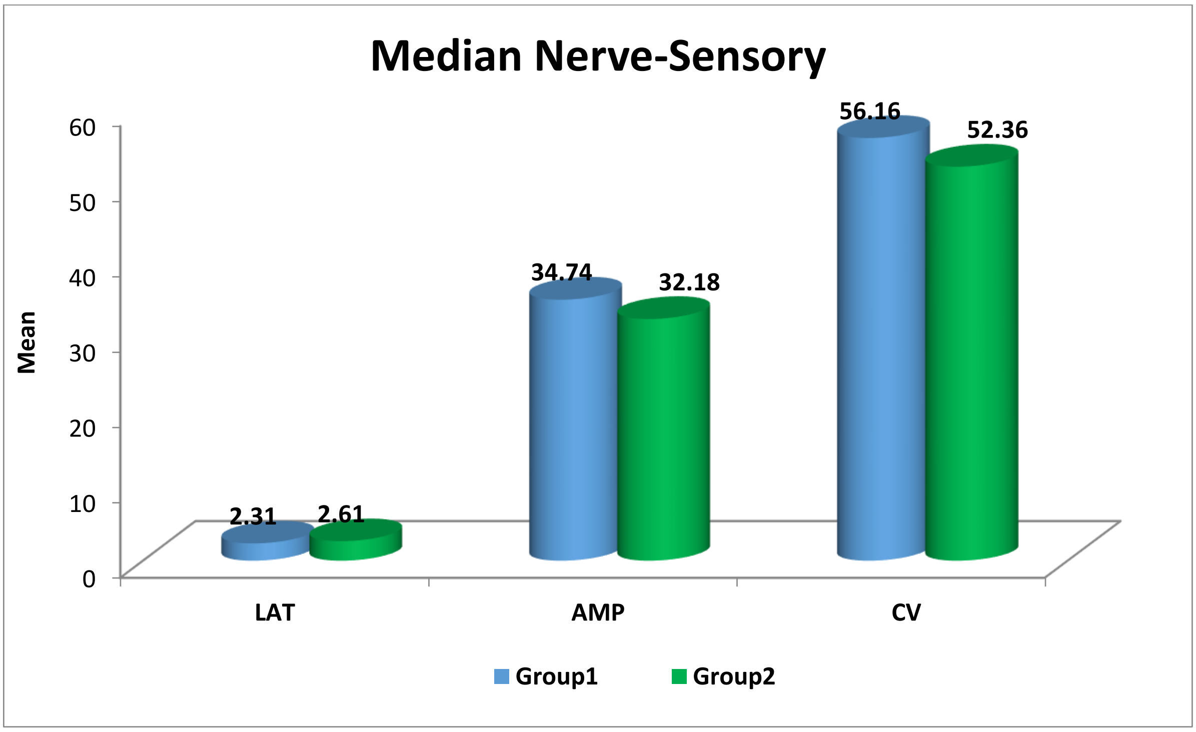


Table 4: Parameters of Median Nerve (Motor) conduction

Median Nerve Motor	Group1 (n=45)		Group2 (n=30)		p value
	Mean(SD)	Range	Mean(SD)	Range	
LAT	2.69(0.5)	1.80 – 3.97	3.14(0.2)	2.67 – 3.45	<0.001*
AMP	5.74(0.5)	4.58 – 7.00	5.61(0.5)	4.35 – 6.86	0.318
CV	59.23(1.7)	57 – 63.45	53.70(2.3)	50.35 – 59.23	<0.001*
FW LAT	22.98(0.4)	22.05 – 24.12	24.53(1.0)	23-26.32	<0.001*

*p <0.05 is statistically significant

Figure: Median-motor nerve conduction parameters between groups

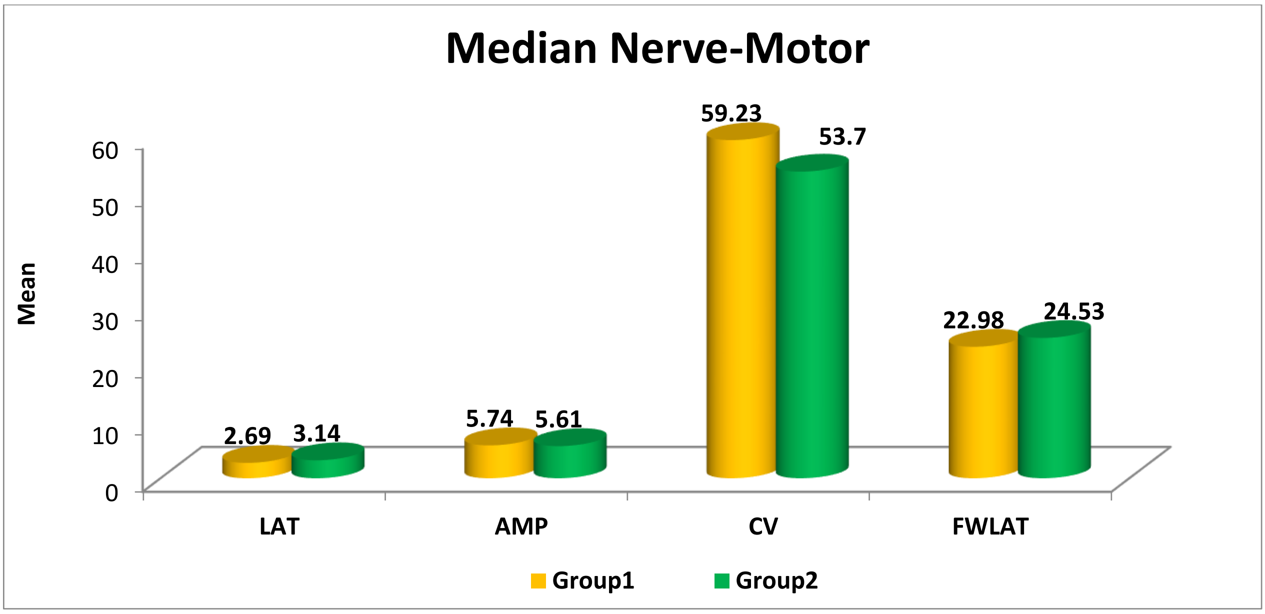


Table 5: Nerve conduction parameters of Sural Nerve (Sensory)

Sural Sensory Nerve	Group1 (n=45)		Group2 (n=30)		p value
	Mean(SD)	Range	Mean(SD)	Range	
LAT	3.09(0.3)	2.39 – 3.98	3.23(0.4)	2.39 – 3.96	0.123
AMP	10.37(1.3)	8.32 – 14.25	8.95(0.7)	7.60 – 10.5	<0.001*
CV	51.22(1.7)	47.21 – 54.5	48.27(1.8)	45.25 – 52.37	<0.001*

*p <0.05 is statistically significant

Figure: Sural nerve (Sensory) conduction parameters between groups

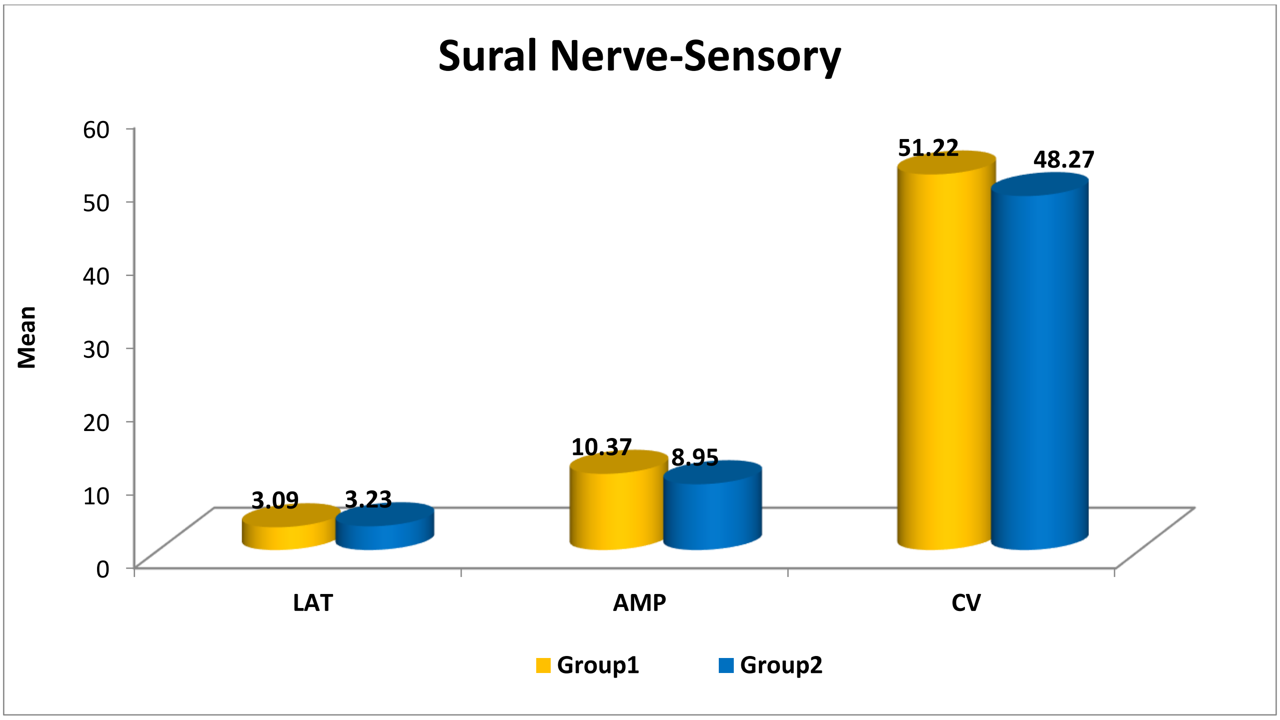
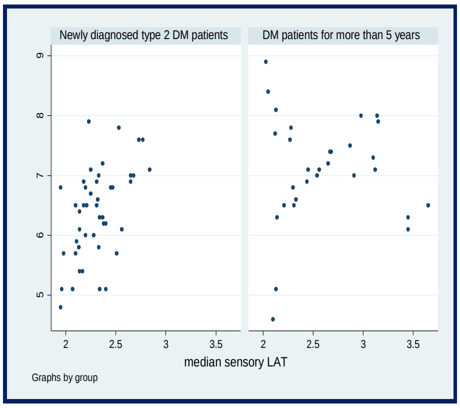


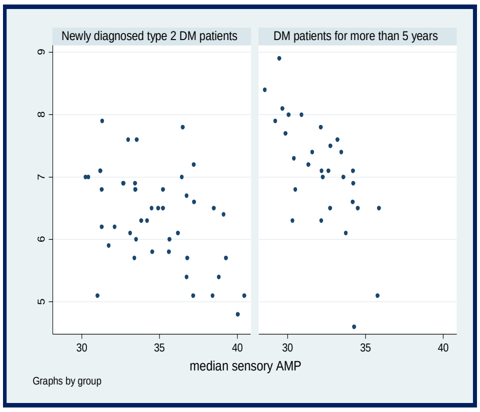
Table 6: Pearson correlation of Median Nerve (Sensory) conduction with HbA1c

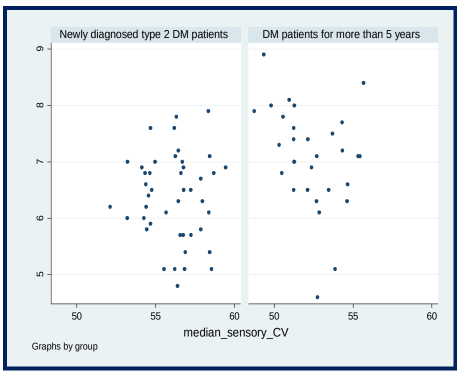
Median Nerve Sensory	Group 1		Group 2	
	Correlation coefficient	p value	Correlation coefficient	p value
LAT	0.5313	<0.001	-0.0261	0.891
AMP	-0.4601	0.002	-0.6915	<0.001*
CV	-0.0429	0.780	-0.3291	0.076

*p <0.05 is statistically significant

<u>SCATTER DIAGRAM</u>







Figures: Pearson correlation of HbA1c with Median nerve (sensory) conduction parameters in two groups

Table 7: Pearson correlation of Median Nerve (Motor) conduction with HbA1c

Median Nerve Motor	Group 1		Group 2	
	Correlation coefficient	p value	Correlation coefficient	p value
LAT	-0.1081	0.479	0.2516	0.180
AMP	-0.1150	0.452	0.0527	0.782
CV	0.0152	0.921	-0.2233	0.236
FW LAT	-0.1408	0.356	0.2439	0.194

Table 8: Pearson correlation of Sural Nerve (Sensory) conduction with HbA1c

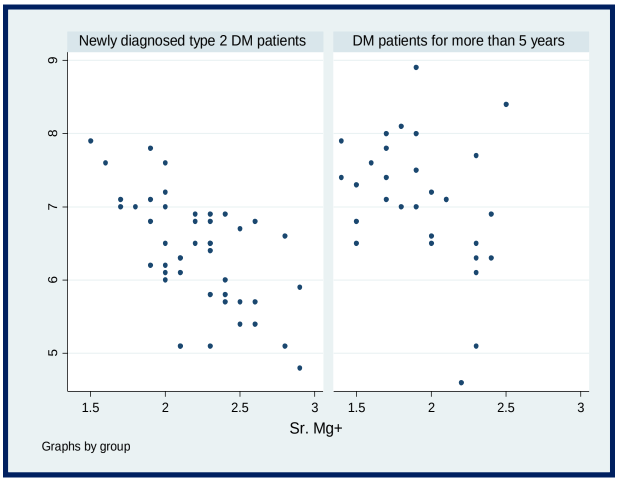
Sural Nerve Sensory	Group 1		Group 2	
	Correlation coefficient	p – value	Correlation coefficient	p value
LAT	0.0179	0.907	0.2361	0.209
AMP	-0.0696	0.650	0.0212	0.912
CV	0.0044	0.977	-0.0967	0.611

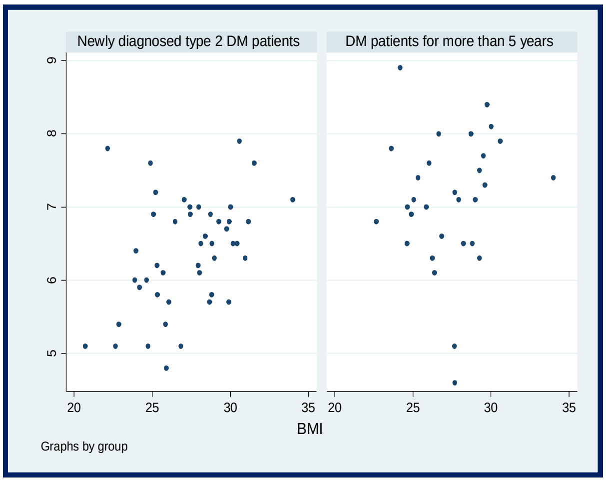
Table 9: Pearson correlation of HbA1c with BMI & Sr. Magnesium

Variable	Group 1		Group 2	
	Correlation coefficient	p value	Correlation coefficient	p value
BMI	0.4015	0.006*	0.0868	0.649
Sr. Mg^{2+}	-0.6058	<0.001*	-0.3482	0.059

*p <0.05 is statistically significant

<u>SCATTER DIAGRAM</u>





Figures: Pearson correlation of HbA1c with BMI and serum magnesium in the two groups

Table 10: Correlation of serum magnesium with nerve conduction parameters in first group

	Median N. Sensory			Median N. Motor				Sural N. Sensory		
	Lat	**Amp**	**CV**	**Lat**	**Amp**	**CV**	**FWLat**	**Lat**	**Amp**	**CV**
Sr. Mg^{2+}	-0.533	0.471	0.094	-0.16	0.313	0.178	-0.093	-0.28	0.029	0.082

Table 11: Correlation of serum magnesium with nerve conduction parameters in second group

	Median N. Sensory			Median N. Motor				Sural N. Sensory		
	Lat	**Amp**	**CV**	**Lat**	**Amp**	**CV**	**FWLat**	**Lat**	**Amp**	**CV**
Sr. Mg^{2+}	-0.27	0.163	0.607	0.217	-0.107	0.232	0.086	-0.071	0.177	0.028

DISCUSSION

- In the first group i.e. newly diagnosed type 2 DM, age distribution ranged from 38 years to 56 years whereas, in the second group the age distribution ranged from 38 years to 65 years. The mean age of group I was 45.6 and group II - 51.27 years. So, this would very clearly elucidate that even though the study population was small it comprised of diabetic patients more in the fourth decade of life. So, type 2 DM is not a disease of old age as previously thought.
- In group I, the female population was little bit higher than male population whereas in group II both sexes were distributed equally. This unequal distribution of sex in group was not done in purpose rather, it happened purely by chance as the female patients who visited the clinic were more compliant. The sample size of the study was fixed to a smaller size as the title under study was a common disorder in the general population. The results of a smaller population would reflect the disease burden of the entire affected population.
- The range of HbA1c in group I was 4.8-7.9 with a mean of 6.37 ± 0.8. The range of HbA1c in group II was 4.6-8.9 with a mean of 7.09 ± 0.9. The means of the two groups were compared and found to be statistically significant with a ‘p’ value <0.001. The mean of HbA1c values for group II was 7.9, a value which crossed the target level of good glycemic control in patients with type 2 DM.

HbA1c was used to diagnose diabetes as early as 1980's, but there were several issues regarding its availability and poor standardization. So, there was hesitancy in using it as the diagnostic tool. In 2009 an international expert committee recommended HbA1c as a diagnostic method for diabetes. It recommended a threshold level of ≥6.5%. This recommendation was adopted by the **American Diabetes Association**[57]and by World Health Organization. HbA1c values indicate chronic hyperglycemia i.e. values for 2-3 months rather than hyperglycemia at a particular point of time. It gives an integrated index of glycemic status of the individual over the entire 120-day lifespan of the red blood cell. The current HbA1c value reflects 50% of HbA1c formed in the month prior to sampling. 25% of HbA1c values reflect the glycosylation that occurred in the month before that. The test's accuracy is affected by conditions that affect red blood cell life span or non-enzymatic glycosylation of hemoglobin. A reduced red blood cell survival time will lower the HbA1c level and may lead to a false negative result. Red blood cell survival time is reduced in hemolytic anemia, chronic renal failure, and severe liver disease, anemia of chronic disease, vitamin B12 and folic acid deficiencies. Patients who undergo regular phlebotomy for medical illnesses like hemochromatosis have false low values. Iron deficiency may also have an impact on red blood cell survival and increase the HbA1c level.

- Serum calcium in group I was 8.0-10.5 mg/dl with a mean of 9.05±0.7 mg/dl. The range of serum calcium in group II was 8.0-10.6 with a mean of 9.07±0.6.The range and mean of both groups were within normal limits and the variations within the normal range between the groups could not be proved statistically significant. So, in our study serum calcium was not correlating with the glycemic control of the subjects.

Nerea Becerra-Tomas et al[58] in their study on 7,447 patients who had any cardiovascular risk factor proved a positive association of increased serum calcium and insulin resistance. It was a longitudinal study which found a link between changes in albumin-adjusted serum calcium concentrations and occurrence of diabetes mellitus. Their results showed that an increase in albumin-adjusted serum calcium levels during follow-up was associated with an increased risk of diabetes. In the study by **Yamaguchi T et al**[59] serum calcium was positively correlating with the fasting plasma glucose in type 2 DM. In the cohort study of **Kim MK et al**[60] abnormal calcium homeostasis proves to have an effect on the occurrence of metabolic syndrome and type 2 DM.

- The range of serum magnesium in group I was 1.5-2.9 mg/dl with a mean of 2.21±0.3. The range of serum magnesium in group II was 1.4-2.5 mg/dl with a mean of 1.94±0.3. On comparing the means of the two groups they were statistically significant with a ‘p’ value of 0.001. This very well proves that in our study as the duration of diabetes mellitus increases the serum magnesium level decrease.

Nehal Hamdy El-said et al[61] in their study “Magnesium in type 2 diabetes mellitus and its correlation with glycemic control” was done to compare serum magnesium levels in type 2 diabetes mellitus patients with non-diabetic healthy control subjects. 60 type 2 diabetic patients and 30 healthy age matched control subjects were enrolled. Fasting blood sugar, fasting insulin, fasting lipids, HbA1C and serum magnesium were measured. Serum magnesium levels were significantly reduced in type 2 DM patients when compared with the control group. After completion of their study they recommended to measure serum magnesium in type 2 DM patients who need supplementation. Case control study by **A.G. Kulkarni et al**[62] showed significant reduction in magnesium levels in type 2 DM when compared with aged matched non-diabetics.

Dana Hyassat et al[63] in their cross-sectional study conducted at the National Center for diabetes, Endocrinology and Genetics in Amman-Jordan performed a comparison of the prevalence of hypomagnesaemia between the studied sample and 3600 individuals enrolled in the National Vitamin D study completed in Jordan in 2009. A total of 1105 patients with type 2 diabetes who attended the center between first of October 2011and end of

February 2012 were included in the study. Out of 1105 patients with type 2 diabetes, 210 patients (19%) were diagnosed with hypomagnesemia. Female gender, hypertension, statin therapy, HbA1c between 7-7.9% or ≥ 9% and patients with diabetes duration more than five years were independent risk factors for hypomagnesaemia. No association between hypomagnesaemia and age distribution, smoking history, neuropathy and retinopathy was found. In comparison with individuals enrolled in the National Vitamin D study, diabetic patients in this study had a much higher prevalence of hypomagnesaemia. Studies by **Ghasemi A et al**[64] and **Arundhati Dasgupta et al**[65] showed that the serum magnesium decreased with poor glycemic control and with increased duration of type 2 DM diabetes mellitus which were similar to the findings present in our study.

- On comparing the mean difference of the latencies of median sensory nerve between two groups, they were statistically significant. The latencies of the median sensory nerve were more prolonged in second group than in the first group. The amplitude and conduction velocity of the nerve is decreased more in the second group. These results indicate us that the duration of type 2 DM had a serious impact on nerve conduction parameters.

These above findings in our study was readily comparable with the results of a study by **Nidhi Yadav et al**[66] in which the diabetes patients had a duration of more than five years duration and the latencies of the nerves were prolonged whereas the amplitude and velocities of the nerves decreased proportionately.

- The analysis of parameters median nerve motor component the distal latency and F-wave latency increased in both groups. The amplitude and conduction velocity decreased in both groups. The distal latency, conduction velocity and F wave latency were statistically significant on comparison of mean difference between the two groups. The decrease in F wave latency was more significant in our study.

Farah Nabil Abbas Al-Sadik's[67] study was on "The Value of Nerve Conduction Study and F-Wave Latency in Subclinical Neuropathic Type II Diabetic Patients". Two subjects groups included in this study were patients with DM and control subjects. The routine nerve conductive study and F-wave were conducted in 35 patients with type II diabetes mellitus but without symptoms and signs of lesions of nervous system. Conduction velocity and sensory amplitude were measured for median, ulnar and sural nerves as well as distal motor latency, motor conduction velocity, motor amplitude and F-wave latency were measured for median, ulnar and tibial nerves in DM patients and their control group. The distal sensory latency of the median nerve was prolonged and the sensory conduction velocity was significantly decreased in diabetic patients (<0.05) when compared with the control group as well as the F-wave of the median nerve was significantly prolonged. There was a significant prolongation of F-wave latency regarding the nerves of both upper and lower extremities. The main advantage of F-wave methodology was in the identification of peripheral neuropathies in which F-wave showed clinically significant and measurable changes even before conventional nerve conduction studies were informative. **Neelamba Prasad et al**[68] conducted a "Comparative analysis of electrophysiological parameters of median nerve in normal and diabetic subjects". Motor nerve conduction study of median nerve was performed on both sides of the body. 40 male diabetes mellitus subjects with relatively short duration of disorder (2.28 ± 1.51 years) were compared with 40 non-diabetes mellitus subjects of same age group, BMI and sex. It was found that motor distal latency of (right and left) median nerves in diabetics was statistically significant when compared with non-diabetics. Results showed a decrease in amplitude and conduction velocities of both median nerves in diabetes mellitus patients. It was statistically significant. Latency was found to be positively correlated with blood sugar. Amplitude, conduction velocity was found negatively correlated with blood sugar.

- In our study, the sural nerve conduction parameters revealed a statistical significance in amplitude and conduction velocity but not in the latency. However, the mean latency was not much increased as expected in the second group and so the comparison of mean difference in latency between the two groups proved to be insignificant. Of the three parameters analyzed the amplitude and velocity of sural nerve was comparable with the other studies.

Marta Banach[69] did a study "To establish the prevalence and type of peripheral neuropathy in diabetic patients by determining the means of nerve conduction studies". A total of 21 patients with type 2 DM presenting with neuropathy symptoms were enrolled in the study. Sensory and motor nerve conduction studies were conducted in the ulnar, median, peroneal, sural, and tibial nerves. The study revealed that 12 patients had nerve conduction abnormalities suggesting peripheral neuropathy and polyneuropathy. A reduction in the amplitude of sensory nerve action potentials (SNAP's) and compound muscle action potentials (CMAP's) in the lower limbs was the most common finding. In addition, their study confirmed that nerve conduction studies are useful in assessing the prevalence of neuropathy and differentiating between axonal and demyelination polyneuropathies. It also emphasized that nerve conduction studies should be part of routine primary care strategies in the management of diabetes mellitus. **Owolabi LF et al**[70] did a case control study entitled “Electrodiagnostic evaluation of median nerve conduction in Type II diabetes mellitus patients that were asymptomatic for peripheral neuropathy”. The study evaluated median nerve conduction of type 2 DM patients who were asymptomatic for neuropathy and to compare their findings with age and sex-matched healthy individuals. On comparison of the various parameters, the median nerve distal latencies and F-responses were significantly higher in the study group while the median nerve conduction velocities and amplitudes were significantly lower in the study group. Thus, early screening for peripheral neuropathy in diabetes was further stressed in their study.

- Pearson correlation analysis of HbA1c with nerve conduction values revealed that the latency of median (sensory) nerve in the first group had a positive correlation with significant ‘p’ value and the amplitude of the same nerve in second group had a negative correlation with significant ‘p’ value.
- Pearson correlation analysis of HbA1c with nerve conduction parameters revealed that latency, F-wave latency, conduction velocity of median (motor)nerve in the second group had a significant correlation with insignificant ‘p’ value. However in the first group except for amplitude all other parameters had an insignificant correlation with HbA1c. Our study revealed that the newly diagnosed

type 2 DM group's latency and amplitude had a significant correlation with HbA1c values. Whereas, in the second group latency and conduction velocity had a significant correlation with HbA1c values.

Rinku Garg et al[71] in their study on sensory and motor conduction of median nerve in asymptomatic type 2 DM subjects had similar results like our study.

- Pearson correlation analysis shown in Table-9 indicates that in both groups HbA1c had an appropriate correlation with BMI and serum magnesium. The second group who had type 2 DM for more than five years had a lower serum magnesium levels with increased HbA1c values and so they are scattered above and to the left in the scatter diagram.
- In Table-10, latency of all nerves was negatively correlated with serum magnesium. The amplitude and conduction velocities of all nerves were positively correlated with serum magnesium. So, serum magnesium seems to be a very good indicator of the effect of diabetes mellitus on peripheral nerves.

So, in our study magnesium had a significant correlation with the glycemic control and with the nerve conduction parameters of the nerves studied. **S.S. Antin et al**[72] had proved magnesium as a cause relating to the complications occurring in type 2 DM. So, magnesium supplementation in type 2 DM as studied by **Lima JDL et al**[73] proved to be useful in preventing the diabetic complications and to keep the blood glucose levels in a check.

SUMMARY AND CONCLUSION

The resistance to insulin-stimulated diabetes is a serious threat to population health.

- This study has brought out the significance of nerve conduction abnormalities in early stages of the disease process. In this study, it was observed that the nerve conduction velocity progressively decreased and latency increased from the first group to second group as duration of diabetes increased. Moreover, it has demonstrated that there is significant impairment of nerve conduction parameters in type 2 DM patients even without subjective features suggestive of peripheral neuropathy. These findings are in accordance with those of previous researchers. Therefore, there is a need for methods to identify the at-risk diabetic patients for neuropathy. Routine nerve conduction studies should be done in diabetics at least on yearly basis.
- This study has given an important clinch about the correlation of serum magnesium with the glycemic status of the individual. So, though it has not been mandatory to do serum magnesium levels as a routine investigation in diabetes mellitus, it is sure an investigation to be done for patients with long-standing diabetes and poorly controlled diabetes mellitus. It is true to the fact that routine surveillance for hypomagnesemia is done and the condition be treated whenever situation occur. A magnesium rich diet consisting of whole grains,

legumes, fruits and vegetables such as spinach, dry apricots may be recommended. Further studies on the role of magnesium supplementation in type 2 DM in our population is recommended.

- HbA1c mean values for the group with longer duration of type 2 DM was higher and it was more than the targeted value for patients on therapy. The poor glycemic control in the second group correlated appropriately with serum magnesium levels. Blood glucose levels begin to have an impact on morbidity and mortality of the subject even before the diagnostic threshold for diabetes is reached. So, information communication and education regarding the modifiable risk factors should be emphasized to the population to achieve the targeted glycemic control.
- In our study, there was no correlation between serum calcium levels and glycemic status or nerve conduction parameters. However, the fact that increased calcium levels can decrease the expression of glucose transporter proteins and consequently, decrease glucose uptake resulting in chronic hyperglycemia should be remembered.

The human and economic costs of this epidemic are enormous. The increasing prevalence of this disorder will inevitably result in increasing proportions of deaths. A united, global initiative is required to address this diabetes epidemic.

FUTURE STUDY PLAN

This study is of public health importance in identifying the earlier diagnosis of neuropathy in type 2 DM. The planned future studies are

- A cohort study with the same parameters and do serum calcium estimation in high risk groups.
- To categorize the population based on their glycemic control and do the correlation of nerve conduction parameters.
- A longitudinal study on a population of type 2 DM by doing serum magnesium levels before and after magnesium supplementation.
- Comparing autonomic function tests in newly diagnosed type 2 DM and DM with duration more than five years.
- Correlation of clinical effects of type 2 DM with age, sex, duration and glycemic control of the patient.

BIBLIOGRAPHY

1. Sarah Wild et al; Global Prevalence of Diabetes-Estimates for the year 2000 and projections for 2030; Diabetes care- volume 27; Number 5- May 2004
2. Global report on Diabetes; WHO Library Cataloguing-in-Publication Data; WHO website (http://www.who.int)
3. Last's Anatomy Regional Applied; 9th Edition: R.M.H. McMinn
4. Basic Histology; Luis Carlos Junqueira, Jose Carneiro , Samsom and Wright; 11th Edition
5. Joslin's Diabetes mellitus, 14th Edition; Ronald Kahn, Gordon C. Weir, George L. King, Alan M. Jacobson, Alan C. Moses, Robert J. Smith
6. R.L.Bijlani- Understanding Medical Physiology – 4th Edition
7. Walter F .Boron & Emile L. Boulpaep -Medical Physiology- Updated 2nd Edition: pages 1075-1092
8. Bruce M. Koeppen, Bruce A Stanton Bern & Levy Physiology -6th Edition: pages 664-666, 676-686
9. Diabetes mellitus-A fundamental and clinical text; Drek LeRoih, Simeon I. Taylor, Jerrold M. Olefsky; 3rd Edition, Chapter 4: pages51,52

10. Vanoye CG et al; The carboxyl terminal of K (ATP) Channels bind nucleotides; J. Biol. Chem. PubMed; 277:23260–23270: (2002)
11. Ganong's Review Of Medical Physiology-Kim E. Barrett, Susan M. Barman, Scott Boitano, Heddwen L. Brooks,- 23rd Edition; pages 315-334
12. G. K. Pal :Text book of medical Physiology; 2nd Edition: pages 396-408
13. Textbook of human physiology; Sarada Subramaniam, K. Madhavan kutty, H.D. Singh; 3rd Edition: pages 538-548
14. Text book of Medical Physiology; Indu Khurana: 2nd Edition; Page774-785
15. Text book of Medical Physiolgy; Guyton & Hall: South Asian Edition; Page 614-622.
16. Best & Taylor's Physiological Basis of Medical Practice; O.P.Tandon & Y. Tripathi et al;13th Ednition: pages 902-919
17. Perl E.;The 1944 Nobel Prize to Erlanger and Gasser; FASEB J. 1994Jul;8(10):782-3.
18. Repetitive discharges from human motor nerves after ischaemia and their absence after cooling; William Cobb and John Marshall; J. neurol. neurosurg. psychiat, 1954, 17, 183

19. The relative excitability and conduction velocity of sensory and motor nerve fibres in man; G. D. Dawson; J Physiol. Feb 28, 1956; 131(2): 436–451
20. Colin D.Binnie et al;Clinical Neurophysiology;1st Edition: pages 1-75
21. A Mallik, Nerve conduction studies; Essentials and pitfalls in practice- J. Neurol. Neurosurg. Psychiatry 2005;76:suppl 2 ii 23 - ii3c
22. Jun Kimura - Principles and Pitfalls of Nerve Conduction Studies- Annals of Neurology Vol 16 No 4 October 1984
23. Dhavalikar- Effect of Skin Temperature on Nerve Conduction Velocity and Reliability of Temperature Correction Formula in Indian Females- Journal of Exercise Science and Physiotherapy; Vol. 5.No.1 ;24-29, 2009
24. James B Caress-Technical, Physiological and Anatomic Considerations in Nerve Conduction Studies; The Clinical Neurophysiology Primer2007, pp 217-227
25. Davidson's Principles and Practice of Medicine; Christopher Haslett, Nicholas A. Boon, Nicki R. Colledge-19th Edition; Pages 646-650
26. Harrison's Principles of Internal Medicine; Congo, Fauci, Kasper, Hauser, Jameson, Loscalzo-18th Edition; Pages 2974-2977

27. De Vos A, Heimberg H et al; Human and rat beta cells differ in glucose transporter but not in glucokinase gene Expression. J. Clin. Invest. 96: 2489-2495, (1995)
28. Tengholm A, and Gylfe E. Oscillatory control of insulin secretion. Mol Cell Endocrinol 297: 58-72, (2009)
29. Nutrition and diet therapy-Evidence based applications; Caroll Lutz and Karen Przytutski; 4th Edition: pages 134, 135
30. Shils, ME;Modern nutrition in Health and Disease;9th Edition Lippincott Williams and Wilkins, Philadelphia,1999
31. Brensilver, JM and Goldberger; A primer of water, electrolytes and acid-base syndromes;8th Edition; FA Davis, Philadelphia
32. Gropper, Smith and Groff: Advanced Nutrition and human metabolism;4th Edition, Wadsworth Blemont , 2005
33. Alzaid A et al; Effects of insulin on plasma magnesium in non-insulin dependent diabetes mellitus: Evidence for insulin resistance; J. Clin. Endocrinol. Metab. 1995;80:1376-1381
34. Use of Glycated Hemoglobin in the Diagnosis of Diabetes Mellitus; Abbreviated Report of a WHO Consultation; WHO / NMH / CHP / CPM / 11.1

35. A hand book on Diabetes mellitus; V.Seshiah, V.S. Ganesan, C.V. Harinarayanan, V. Balaji, Madhuri balaji; 2nd Edition
36. Robbins & Cotran Pathologic basis of Disease-South Asia Edition; Pages 1106-1108,1111-1118
37. Chandrashekar M Sultanpur et al; Comprehensive Review On Hba1c In Diagnosis Of Diabetes Mellitus; International Journal of Pharmaceutical Sciences Review and Research: Volume 3, Issue 2, July – August 2010; Article 021
38. Firas Salih Abdul Hadi & Ali Shalash Sultan; The effects of type 2 diabetes mellitus on the levels of glycated hemoglobin, testosterone, leptin and calcium in Iraqi male patients; Global journal of bio-science and bio-technology, Vol.5 (2) 2016: 164-170
39. Rajni Dawar Mahajan et al; Using Glycated Hemoglobin HbA1c for diagnosis of Diabetes mellitus-An Indian perspective; Int. J. Biol. Med. Res. 2011; 2(2) 508-512
40. Chris Florkowski; HbA_{1c} as a Diagnostic Test for Diabetes Mellitus – Reviewing the Evidence; Clin Biochem Rev. 2013 Aug; 34(2): 75–83
41. Zaccardi F et al; Association between direct measurement of active serum calcium and risk of type 2 diabetes mellitus- a prospective study; Diabetes Research Centre, University of Leicester, Leicester, UK;

Nutrition, Metabolism & Cardiovascular Diseases 2015 Jun;25(6):562-8. doi: 10.1016/j.numecd.2015.02.013. PMID:25933474.

42. Kanchana N Saikumar P; Serum Calcium levels in Type 2 Diabetes Mellitus; IOSR Journal of Dental and Medical Sciences Volume 13, Issue 8 Ver. II (Aug. 2014), PP 01-03

43. D.S. Pushparani, S. Nirmala; High level of serum calcium and iron influences the risk of type 2 diabetes mellitus with periodontitis; Journal of Asian Scientific Research, 2014, 4(2): 70-82

44. Anubha Shukla et al; Involvement of Calcium and Vitamin C in Type 2 DM; IOSR Journal of Pharmacy Vol. 2, Issue 1, Jan-Feb.2012, pp. 009-020

45. Mohamed Murtuza Kauser et al; Study of Serum Magnesium in Type 2 Diabetes Mellitus and its Correlation with the Modality of Treatment-A South Indian Study; Global Journal of Medical research: Vol.14 Issue 4 2014

46. Prabhu G et al; A study of serum magnesium level in type 2 diabetes mellitus and its significance; International Journal of Medical Research and Review August, 2015/ Vol. 3/Issue 7

47. Pramod P Rao, Mohamed Ghouse Shariff; Serum Magnesium Levels in Type 2 Diabetic Patients with Microalbuminuria and

Normoalbuminuria; International Journal of Scientific Study, July 2015 Vol. 3 Issue 4

48. Sasmita Mishra et al; Serum Magnesium and Dyslipidemia in Type-2 Diabetes Mellitus**;** Biomedical Research 2012; 23 (2): 295-300

49. Dr. K. Sumathi, Dr. A. Mary Chandrika; Role of magnesium in pre diabetes; Sch. Acad. J. Pharm., 2015; 5(1): 12-13

50. S. Ramadass et al; Serum magnesium levels as an indicator of status of Diabetes Mellitus type 2;Diabetes & Metabolic Syndrome: Clinical Research & Reviews, journal homepag e: www .elsevier .com /locate/dsx

51. Abhijeet A. Adgaonkar et al; Clinical Profile of Peripheral Neuropathy in Diabetes Mellitus by Nerve Conduction Study; Scholars Journal of Applied Medical Sciences; 2014: 2(6A):1973-1977

52. Yacoub G. Bahou et al; A clinical and electrodiagnostic study of diabetic neuropathy at Jordan University Hospital; 5A clinical – 20061514: 216

53. Arindam Dutta et al; Prevalence of peripheral neuropathy in newly diagnosed type 2 diabetics; Int. J. Diab. Dev. Countries (2005), Vol. 25

54. Kanavi Roopa Shekharappa et al; A study on the Utility of Nerve Conduction studies in type 2 DM; Journal of Clinical and Diagnostic Research: 2011 June, Vol-5(3): 529-531
55. Vijay Viswanathan et al; Nerve conduction abnormalities in different stages of glucose Intolerance; Neurology India December 2004 Vol. 52 Issue 4
56. U K Misra and Khalida -Clinical Neurophysiology-2nd Edition; pages 21-36, 107-110
57. American Diabetes Association; Standards of medical care in diabetes; Diabetes Care 2013; 36(Suppl. 1):S11–S66
58. Nerea Becerra-Tomas et al; Increased Serum Calcium Levels and Risk of Type 2 Diabetes in Individuals at High Cardiovascular Risk; Diabetes Care Volume 37, November 2014
59. Yamaguchi T et al; Correlation of serum calcium with fasting plasma glucose and insulin resistance male patients with type 2 diabetes mellitus; Metabolism 2011;60:1334–1339
60. Kim MK, Kim G, Jang EH, et al; Altered calcium homeostasis is correlated with the presence of metabolic syndrome and diabetes in middle-aged and elderly Korean subjects; the Chungju Metabolic

Disease Cohort study (CMC study). Atherosclerosis 2010;212; 674–681

61. Nehal Hamdy El-said et al; Magnesium in type 2 diabetes mellitus and its correlation with glycemic control; International Journal of Research in Medical Sciences August 2015 Vol 3. Issue 8 Page 1958

62. A.G.Kulkarni et al; Study of serum magnesium level in Type 2 Diabetes Mellitus , IOSR Journal of Dental and Medical Sciences; Volume 13, Issue 4 Ver. VII. (Apr. 2014), Pages 115-119

63. Dana Hyassat et al; Prevalence of Hypomagnesaemia among Obese Type 2 Diabetic Patients Attending the National Center for Diabetes, Endocrinology and Genetics; Int. J. Endocrinol. Metab. 2014 July. 12(3): e17796

64. Ghasemi A et al; Low serum magnesium levels in elderly subjects with metabolic syndrome; Biol. Trace Elem. Res. 2010; 136(1):18–25.

65. Arundhati Dasgupta et al; Hypomagnesemia in type 2 diabetes mellitus; Indian J. Endocrinol. Metab. 2012; 16(6):1000-1003.

66. Nidhi Yadav et al; Study Of Nerve Conduction Velocity In Type II Diabetes Mellitus; NJIRM 2015; Vol. 6(4) July – August

67. Farah Nabil Abbas Al-Sadik; The Value of Nerve Conduction Study and F-Wave Latency in Subclinical Neuropathic Type II Diabetic Patients; Medical Journal of Babylon-Vol. 9- No. 4 -2012
68. Neelambala Prasad et al; Comparative Analysis of Electrophysiological Parameters of Median Nerve in Normal and Diabetic Subjects; Indian Medical Gazette — July 2013: page 261
69. Marta Banach; The utility of nerve conduction studies in patients with diabetic polyneuropathy; Family Medicine & Primary Care Review 2015; 17, 3: 171–174
70. Owolabi LF et al; Electro diagnostic evaluation of median nerve conduction in Type II diabetes mellitus patients that were asymptomatic for peripheral neuropathy-a case control study; International Journal of Medicine and Biomedical Research Volume 4 Issue 3 September – December 2015
71. Rinku Garg et al; A Study of Median Nerve Conduction Velocity in Diabetes Mellitus Type 2 in Neurologically Asymptomatic Patients; International Journal of Health Sciences & Research; Vol.3; Issue: 5; May 2013
72. Dr.S.S.Antin et al**;** A Cross Sectional Study of Fasting Serum Magnesium Levels in the Patients with Type 2 Diabetes Mellitus and

Its Relation to Diabetic Complications; Scholars Journal of Applied Medical Sciences**,** 2014; 2(2A):502-506

73. Lima JDL et al; The effect of magnesium supplementation in increasing doses on the control of type 2 diabetes; Diabetes care:1998;21:682-686

INFORMED CONSENT FORM

Study Title A STUDY TO CORRELATE SERUM MAGNESIUM, SERUM CALCIUM, HbA1c AND NERVE CONDUCTION PARAMETERS IN TYPE 2 DIABETES MELLITUS

Address ____

1. I confirm that I have read and understood the information sheet dated for the above study and have had the opportunity to ask questions.
   OR I have been explained the nature of the study by the Investigator and had the opportunity to ask questions
2. I understand that my participation in the study is voluntary and that I am free to withdraw at any time, without giving any reason and without my medical care or legal rights being affected.
3. I understand that the sponsor of the clinical trial/project, others working on the Sponsor's behalf, the Ethics Committee and the regulatory authorities will not need my permission to look at my health records both in respect of the current study and any further research that may be conducted in relation to it, even if I withdraw from the trial. However, I understand that my Identity will not be revealed in any information released to third parties or published.
4. I agree not to restrict the use of any data or results that arise from this study provided such a use is only for scientific purpose(s)
5. I agree to take part in the above study

Signature (or Thumb impression) of the Subject/Legally Acceptable Representative: __________________

Signatory's Name ____ Date ____________________________

Signature of the Investigator _______________________________ Date ____________________________

Study Investigator's Name __________________________________

Signature of the Witness __________________________________ Date ____________________________

Name of the Witness

நோயாளிகளுக்கு அறிவிப்பு மற்றும் ஒப்புதல் படிவம்
(மருத்துவஆய்வில் பங்கேற்பத்ற்கு)

ஆய்வு செய்யப்படும் தலைப்பு:
பங்கு பெறுவரின் பெயர்:
பங்கு பெறுவரின் வயது:

		பங்கு பெறுவர் இதனை ✓ குறிக்கவும்
1.	நான் மேலேகுறிப்பிட்டுள்ள மருத்துவ ஆய்வின் விவரங்களை படித்து புரிந்துகொண்டேன். என்னுடைய சந்தேகங்களை கேட்கவும், அதற்கான தகுந்த விளக்கங்களை பெறவும் வாய்ப்பளிக்கப்பட்டுள்ளுத என அறிந்துகொண்டேன்.	☐
2.	நான் இவ்வாய்வில் தன்னிச்சையாக தான் பங்கேற்கிறேன். எந்தகாரணத்தினாலோ எந்தகட்டத்திலும் ,எந்தசட்டசிக்கலுக்கும் உட்படாமல் நான் இவ்வாய்வில் இருந்து விலகிகொள்ளலாம் என்றும் அறிந்துகொண்டேன்.	☐
3.	இந்த ஆய்வு சம்பந்தமாகவோ, இதைசார்ந்துமேலும் ஆய்வுமேற்காள்ளும் போதும் இந்த ஆய்வில் பங்கு பெறும் மருத்துவர் என்னுடைய மருத்துவ அறிக்கைகளை பார்ப்பதற்குஎன் அனுமத ிதேவையில்லைஎன அறிந்துகொள்கிறேன். நான் ஆய்வில் இருந்துவிலகிக் கொண்டாலும் இது பொருந்தும் என அறிகிறேன்.	☐
4.	இந்த ஆய்வின் மூலம் கிடைக்கும் தகவலையோ,முடிவையோ பயன்படுத்திக் கொள்ள மறுக்கமாட்டேன்.	☐
5.	இந்த ஆய்வில் பங்கு கொள்ள ஒப்புக் கொள்கிறேன் எனக்கு கொடுக்கப்பட்டஅறிவுரைகளின் படி நடந்து கொள்வதுடன், ஆய்வை மேற்கொள்ளும் மருத்துவ அணிக்கு உண்மையுடன் இருப்பேன் என்று உறுதியளிக்கிறேன். என் உடல் நலம் பாதிக்கப்பட்டாலோ, அல்லது எதிர்பாராத, வழக்கத்திற்கு மாறான நோய்குற ிதென்பட்டாலோ உடனே இதை மருத்துவ அணியிடம் தெரிவிப்பேன் என உறுதி அளிக்றேன்.	☐

பங்கேற்பவரின் கையொப்பம் / .... இடம் ....

கட்டைவிரல் ரேகை

பங்கேற்பவரின் பெயர் மற்றும் விலாசம் ....

ஆய்வாளரின் கையொப்பம் /... இடம் ....

ஆய்வாளரின் பெயர்..

மையம் ....

கல்வியறிவு இல்லாதவற்கு (கைரேகைவைத்தவர்களுக்கு) இது அவசியம் தேவை

சாட்சியின் கையொப்பம் /.. இடம் ....

பெயர் மற்றும் விலாசம் ....

PROFORMA

NAME: O.P no IP no

AGE/SEX: Date

ADDRESS:

OCCUPATION/ EDUCATION:

HISTORY:

1. Duration of diabetes
2. Treatment particulars OHA/Insulin Regular/Irregular
3. Any other disorders
   - Anemia
   - Thyroid disorders
   - Hypertension
   - Tuberculosis
   - Epilepsy
   - Neuromuscular disorders
   - Any other illness
4. Other medications
5. History of smoking/Alcohol
6. Family history of neuropathy
7. History of symptoms of peripheral neuropathy

ANTHROPOMETRIC EXAMINATION:

Height in cm

Weight in kg

Body mass index

CLINICAL EXAMINATION:

General examination:

Anaemia ,

Icterus

Pedal oedema

Blood pressure   in mm Hg

Systemic examination:

Respiratory system

Cardiovascular system

Central nervous system

If, any other details of illness

INVESTIGATIONS:

HbA1c

Serum calcium

Serum magnesium

NERVE CONDUCTION STUDY DETAILS:

Group I : Type 2 DM patients with duration less than 1 year																			
SL. No.	Sex	Age (yrs)	Wt (kgs)	Ht (mts)	BMI	HbA1c	Sr. Ca	Sr. Mg	MEDIAN N. SENSORY			MEDIAN N. MOTOR				SURAL N. SENSORY		
									LAT ms	AMP µV	CV m/s	LAT ms	AMP mV	CV m/s	FW LAT	LAT ms	AMP µV	CV m/s
1	F	43	58	1.48	26.48	6.8	9.5	2.3	1.95	33.45	54.64	2.85	5.67	58.46	23.34	3.1	10.8	50.43
2	F	45	67	1.49	30.18	6.5	8.3	2	2.18	38.49	54.75	3.1	5.89	58.69	23.15	3.2	9.91	51.12
3	F	41	65	1.54	27.41	7	9.1	1.7	2.33	36.45	53.21	3	4.68	57.15	23.57	3.17	11.23	49.88
4	F	47	59	1.53	25.21	7.2	9.3	2	2.37	37.2	56.43	2.94	6.07	59.34	22.45	3.05	12.21	52.37
5	F	42	53.2	1.49	23.96	6.4	9.2	2.3	2.14	39.12	54.56	2.15	5.9	58.75	23.56	2.39	9.85	51.44
6	F	40	68.5	1.5	30.44	6.5	8.1	2.3	2.1	35.23	56.78	2.88	6.1	59.47	23.14	3.1	10.21	52.35
7	F	42	70	1.58	28.04	6.1	8.5	2	2.14	33.12	55.67	3.8	4.89	57.27	24.12	3.7	12.32	51.97
8	F	45	71	1.54	29.94	6.8	9	1.9	2.47	31.28	54.32	3.12	5.05	58.1	22.1	3.17	9.43	49.55
9	F	47	69	1.57	27.99	7	8.6	1.8	2.65	30.43	54.97	3.97	5.48	58.48	22.58	3.98	10.25	49.65
10	F	49	55.3	1.49	24.9	7.6	8.8	2	2.73	33	54.67	2.94	5.39	58.23	22.64	3.1	10.67	49.43
11	F	41	67.3	1.5	29.91	5.7	8.4	2.5	1.98	33.4	56.55	2.58	5.88	58.45	22.98	2.66	9.57	52.38
12	F	40	68	1.54	28.67	5.7	8.3	2.4	2.1	36.78	57.25	2.64	6.34	62.36	22.5	2.85	9.45	53.1
13	F	48	72	1.52	31.16	6.8	8.9	2.2	2.2	35.23	58.68	1.97	6.9	63.45	22.68	2.62	9.21	53.27
14	F	39	65.7	1.51	28.81	5.8	9.2	2.3	2.13	34.54	57.86	2.14	5.98	60.48	23.14	2.88	9.34	52.75
15	F	46	67.8	1.48	30.95	6.3	10	2.1	2.34	34.21	56.43	2.65	5.5	57.78	23.67	2.85	9.12	51.34
16	F	41	61.5	1.58	24.64	6	8.6	2.4	2.28	35.65	53.2	2.32	5.71	57.8	22.56	2.98	10.2	47.21
17	F	40	60	1.42	29.76	6.7	8.2	2.5	2.25	36.75	57.86	2.1	6.82	61.5	23.18	2.57	9.27	53.25
18	F	44	73.5	1.55	30.6	7.9	8.1	1.5	2.23	31.32	58.34	1.8	7	63.24	22.9	2.78	9.05	54.32
19	F	40	51	1.5	22.67	5.1	8.5	2.8	2.07	38.43	56.21	2.32	5.47	58.35	23.54	2.67	8.95	50.43
20	F	48	71.5	1.45	34	7.1	8	1.9	2.25	31.2	58.43	2.12	5.95	59.14	23.25	3.1	9.65	53.65
21	F	45	64	1.49	28.83	6.5	9.3	2.3	2.21	34.5	57.23	2.56	5.38	58.4	22.88	2.98	10.34	51.35
22	F	47	60.4	1.47	27.95	6.2	9.4	2	2.38	32.13	52.12	2.95	5.19	57.16	23.15	3.24	11.25	47.49
23	F	46	53	1.48	24.2	5.9	9	2.9	2.11	31.74	54.67	3.25	5.12	57.88	23.18	3.28	10.76	50.56
24	F	44	61.2	1.51	26.84	5.1	8.7	2.3	1.96	37.16	58.55	2.68	6.1	60.5	22.15	3.12	9.85	52.36
25	F	43	67	1.52	28.99	6.3	8.2	2.1	2.37	33.82	57.97	2.26	6.27	60.38	22.34	2.88	8.85	53.12
26	M	40	70.3	1.53	30.03	7	9.8	2	2.68	30.25	56.7	2.56	5.38	59	23.1	3.09	10.33	50.64
27	M	49	64.6	1.58	25.87	5.4	10	2.5	2.14	36.75	58.43	2.65	5.87	59.38	22.9	2.97	12.34	52.43
28	M	48	59.3	1.53	25.33	5.8	9.5	2.4	2.33	35.61	54.43	2.61	5.75	58.18	23.17	2.91	10.33	49.49
29	M	38	62.4	1.46	29.27	6.8	9.8	2.6	2.45	33.45	56.61	2.13	6.45	62.85	22.05	3.12	9.62	50.25

Group I : Type 2 DM patients with duration less than 1 year

SL. No.	Sex	Age (yrs)	Wt (kgs)	Ht (mts)	BMI	HbA1c	Sr. Ca	Sr. Mg	MEDIAN N. SENSORY			MEDIAN N. MOTOR				SURAL N. SENSORY		
									LAT ms	AMP µV	CV m/s	LAT ms	AMP mV	CV m/s	FW LAT	LAT ms	AMP µV	CV m/s
30	M	47	65.5	1.51	28.73	6.9	11	2.4	2.18	33.43	59.43	2.25	6.08	62.19	22.36	2.83	10.33	54.26
31	M	56	68.5	1.56	28.12	6.5	9.7	2.2	2.31	34.91	56.78	2.46	5.82	60.5	22.54	2.99	9.96	50.98
32	M	48	64	1.59	25.31	6.2	10	1.9	2.4	31.28	54.4	3.12	5.1	59.23	23.1	3.59	8.32	49.27
33	M	51	60	1.62	22.86	5.4	8.2	2.6	2.17	38.82	56.88	2.15	6.23	61.6	22.65	3.1	9.15	50.48
34	M	43	66.5	1.53	28.41	6.6	9.2	2.8	2.32	37.23	54.38	2.48	5.88	59.25	22.87	2.97	8.75	49.91
35	M	49	67.5	1.58	27.04	7.1	9.8	1.7	2.84	31.2	56.25	3.89	4.96	57	23.55	3.96	9.35	50.81
36	M	50	63.4	1.52	27.44	6.9	8.9	2.2	2.65	32.67	54.12	2.68	5.49	57.13	23.24	3.19	13.25	49.13
37	M	46	71.5	1.7	24.74	5.1	8.7	2.1	2.34	40.45	56.83	2.75	5.38	58.45	23.12	3.17	12.84	50.65
38	M	48	68.4	1.62	26.06	5.7	9.2	2.6	2.51	39.27	56.75	2.8	5.78	58.71	23.1	3.02	11.55	51.48
39	M	50	70	1.65	25.71	6.1	11	2.1	2.56	36.18	58.37	3.68	6.12	59.67	22.97	3.65	13.69	53.45
40	M	52	65.5	1.59	25.91	4.8	9	2.9	1.95	40.03	56.38	3.56	6.24	59.28	23.25	3.72	9.88	54.5
41	M	42	74.8	1.54	31.54	7.6	8.2	1.6	2.77	33.54	56.18	2.57	5.12	58.5	23.17	3.12	9.16	51.43
42	M	48	79.5	1.78	25.09	6.9	8.6	2.3	2.31	32.71	56.77	2.5	5.86	58.9	22.68	2.96	14.25	50.82
43	M	49	60.4	1.59	23.89	6	9.5	2	2.2	33.51	54.26	2.49	5.75	59.15	23.54	2.99	11.1	49.1
44	M	54	57.8	1.67	20.72	5.1	9.8	2.1	2.4	31.02	55.53	2.63	5.66	58.38	23.23	3.11	10.91	49.77
45	M	51	59.6	1.64	22.16	7.8	8.7	1.9	2.53	36.5	56.32	2.16	4.58	57.2	22.65	3.19	9.93	51.23

BMI- Body Mass Index

HbA1c- Glycosylated hemoglobin

Sr.Ca - Serum Calcium in mg/dl

Sr. Mg- Serum Magnesium in mg/dl

LAT - Latency Measured in milliseconds

AMP- Amplitude Measured in Millivolts for motor and in micro volts for sensory nerves

CV- conduction Velocity Measured in meter per seconds

FW LAT- F wave Latency Measured in Milli Seconds

Group II : Type 2 DM Patients with duration more than 5 years

SL. No.	Sex	Age (yrs)	Wt(kgs)	Ht(mts)	BMI	HbA1c	Sr. Ca+	Sr. Mg+	MEDIAN N. SENSORY			MEDIAN N. MOTOR				SURAL N. SENSORY		
									LAT ms	AMP μV	CV m/s	LAT ms	AMP mV	CV m/s	FW LAT	LAT ms	AMP μV	CV m/s
1	F	54	59	1.58	23.63	7.8	9.3	1.7	2.28	32.12	50.56	3.13	5.21	57.23	25.23	3.56	7.8	47.84
2	F	53	61	1.53	26.05	7.6	8.2	1.6	2.27	33.2	51.25	3.15	6.23	54.35	24.97	3.54	7.91	48.95
3	F	65	60	1.5	26.67	8	9	1.9	2.98	30.9	49.8	3.24	5.65	56.75	25.56	3.49	7.6	47.85
4	F	60	64	1.52	27.7	7.2	8.2	2	2.65	31.32	54.33	3.31	6.15	58.72	25.68	3.25	8.13	52.37
5	F	59	52	1.44	25.07	7.1	8	2.1	2.45	34.2	55.45	2.98	5.45	59.23	23.56	3.68	9.1	50.78
6	F	62	67	1.54	28.25	6.5	8.8	2	2.31	35.86	52.12	2.67	5.95	55.67	23.14	3.29	8.47	51.89
7	F	54	61	1.52	26.4	6.1	8.8	2.3	3.45	33.75	52.85	3.45	4.89	57.18	26.32	3.85	9.54	46.83
8	F	58	60	1.56	24.65	7	9.1	1.9	2.91	32.25	51.28	3.14	5.12	54.21	24.92	3.12	9.13	47.25
9	F	61	73	1.57	29.62	7.3	8.5	1.5	3.1	30.4	50.32	3.29	4.35	54.32	25.18	2.97	8.28	47.1
10	F	63	55.3	1.49	24.91	6.9	8.9	2.4	2.44	34.21	52.38	3.43	5.18	53.2	25.64	2.58	9.63	48.83
11	F	57	67.3	1.51	29.52	7.7	9	2.3	2.12	29.85	54.32	3.12	5.16	52.69	24.98	3.54	9.13	49.14
12	F	52	59	1.46	27.67	5.1	8.4	2.3	2.13	35.78	53.87	2.89	5.24	53.25	23.5	2.86	8.25	50.45
13	F	51	74	1.59	29.27	6.3	8.8	2.4	3.45	30.31	54.62	3.42	6.86	54.75	25.87	3.52	9.15	47.81
14	F	49	65.7	1.54	27.7	4.6	9.4	2.2	2.1	34.26	52.75	2.95	6.12	55.65	23.14	2.78	9.12	45.5
15	F	55	58.3	1.49	26.26	6.3	8.6	2.3	2.14	32.16	52.68	3.17	5.43	54.1	23.67	3.96	8.13	47.92
16	M	50	61.5	1.58	24.64	6.5	9.8	2.3	2.21	32.73	51.25	2.97	5.32	53.48	23	2.54	9.22	46.66
17	M	47	60	1.42	29.75	8.4	8.9	2.5	2.05	28.54	55.67	3.42	5.79	51.25	24.19	3.12	9.95	45.25
18	M	44	73.5	1.55	30.59	7.9	10.2	1.4	3.15	29.2	48.75	3.11	6.15	52.35	23.86	2.99	8.45	47.28
19	M	40	51	1.5	22.67	6.8	9	1.5	2.3	30.5	50.5	3.25	6.23	53.18	24.54	3.28	10.12	49.33
20	M	48	71.5	1.45	34	7.4	9.6	1.4	2.68	31.59	51.24	2.92	5.97	52.5	23.25	3.15	9.43	47.25
21	M	45	64	1.49	28.82	6.5	8.8	1.5	3.65	34.51	53.45	2.95	5.88	51.54	23.88	2.39	8.17	50.45
22	M	47	60.4	1.47	27.95	7.1	9.3	1.7	2.56	32.18	52.71	3.13	5.5	51.36	24.19	3.88	9.26	49.15
23	M	46	53	1.48	24.2	8.9	8.9	1.9	2.03	29.45	49.35	3.19	6.25	52.35	24.96	3.52	8.75	49.25
24	M	44	61.2	1.51	26.84	6.6	10.6	2	2.33	34.18	54.65	3.24	5.12	51.33	25.12	2.59	9.56	50.05
25	M	43	67	1.52	28.99	7.1	9.2	2.1	3.12	32.61	55.32	3.28	5.23	53.71	25.64	3.57	8.82	49.25
26	M	49	70.3	1.53	30.03	8.1	10.4	1.8	2.13	29.65	50.95	3.15	5.17	52.95	24.99	3.22	9.05	46.85
27	M	49	64.6	1.58	25.87	7	9.4	1.8	2.54	33.57	51.27	2.78	5.25	51.23	23.65	3.47	8.5	45.5
28	M	48	59.3	1.53	25.33	7.4	9	1.7	2.67	33.45	52.13	3.41	5.68	50.45	26.12	2.55	9.35	47.14
29	M	38	62.4	1.46	29.27	7.5	9.1	1.9	2.87	32.75	53.69	2.93	6.12	51.65	23.16	2.98	10.5	46.28
30	M	47	65.5	1.51	28.73	8	8.8	1.7	3.14	30.05	51.28	3.15	5.67	50.35	23.91	3.61	9.86	47.88